# Effect of Some Plant growth promoting rhizobacteria (PGPR) on growth of *Piper nigra*

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**ABSTRACT:** Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that aggressively colonize plant roots and enhance plant growth by a wide variety of mechanisms such as phosphate solubilization, phytohormone production, antifungal activity, etc. In this study, effect of plant growth promoting rhizobacteria (PGPR) on *Piper nigra* 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 *Piper nigra* seeds. The growth parameters (plant height, stem width root length and the internode of the plant) were monitored at 5 days after planting (DAP) 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 combined use of the three isolates gave the best performance in terms of enhanced increases in growth parameters than the control (treatment A). Thus, the use of combined biofertilizers is advocated for excellent growth performance of plants.

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## 1. INTRODUCTION

Black pepper is native to South India and is extensively cultivated there and elsewhere in tropical regions. Black pepper (*Piper nigrum*) is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as an spice and seasoning (Dastager et al., 2011). It grows successfully between 20$^{o}$ North and South latitude and up to 1500 meters above the sea level. The crop tolerates temperature between 10$^{o}$ and 40$^{o}$C (Dastager et al., 2011).

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and promote growth when added to seeds, roots or tubers have been termed plant-growth-promoting rhizobacteria and increase plant growth and yield (Wu et al., 2005). Plant growth-promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Rodríguez et al., 2006). The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields (Rodríguez et al., 2006). Nitrogen (N) and Phosphorus (P) is a vital nutrient required for the growth and development of both plants as well as microorganisms (Vikram, 2007). Considerable work on phosphate solubilizing microorganisms has been done earlier and the results invariably indicated that inoculation of crops with phosphate solubilizing bacteria improved growth and increased the yield and P uptake in a variety of crop plants (Hameeda *et al*., 2006a; Vikram, 2007).

Inoculation of ornamentals, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes (Saharan and Nehra, 2011). PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms (Saharan and Nehra, 2011). The mechanisms by which PGPRs promote plant growth are not fully understood (Vessy, 2003). But, several mechanisms have been suggested by which PGPR can promote plant growth, including phytohormone production (Shaharoona et al., 2006; Egamberdiyeva, 2007), enhancing stress resistance, asymbiotic $N_2$ fixation (Salanture *et al*., 2006), stimulation of nutrient uptake and biocontrol of pathogenic microorganisms (Rodriguez and Fraga., 1999), increasing the supply or availability of primary nutrients to the host plant (Wu et al., 2005), the synthesis of antibiotics, enzymes and fungicidal compounds (Bharathi et al., 2004; Jeun et al., 2004;

Ahmad et al., 2006) and also solubilisation of mineral phosphates and other nutrients (Nezarat and Gholami, 2009). Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins (Vikram *et al*., 2007), cytokinins (Arkhipova et al., 2005) and gibberellins (Joo et al., 2004) produced by rhizobacteria can influence plants growth, including root development which improve uptake of essential nutrients thus increasing plant growth (Vikram *et al*., 2007). Indirect growth promotion occurs through the elimination of pathogens by the production of cyanide, siderophores (Suresh et al., 2010), siderophores, protease, antimicrobials, phosphate solubilizing enzymes (Chaiharn et al., 2008). These may vary with the edaphic conditions (Abbas et al., 2009).

Biofertilizers like phosphate solubilizing microbes, mycorrhizae, *Azospirilum* sp, *Azotobacter* sp, *Rhizobium* sp, *Nitrosomonas* sp, and *Nitrobacter* sp have been in use for a very long time. 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). Thus, the aim of this study was to determine the effect of biofertilizers on the growth of *Piper nigra* plant.

## 2. MATERIALS AND METHOD

### 2.1. COLLECTION OF SOIL SAMPLE

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

### 2.2. ISOLATION, PURIFICATION AND IDENTIFICATION OF ISOLATES

The nitrogen fixing organisms were isolated from the soil sample using the spread plate method. 5g of soil sample was mixed thoroughly in sterile physiological saline of 50ml. 1ml of the suspension was aseptically with drawn with a sterile pipette and serially diluted up to $10^{-6}$ using the ten fold serial dilution technique. After the serial dilution, 0.1ml of each dilution ($10^{-1}$-$10^{-6}$) was inoculated onto duplicate set of the various enrichment media. Plates were then incubated at $37^0$C and examined after 7days for growth. The colonies that developed were picked based on morphological characteristics. Each colony picked was subcultured into the respective enrichment media using a sterile wire loop under aseptic conditions to streak onto the duplicate plates and incubated at $37^0$C for 24-48hours. After incubation, the cultures were sub-cultured to get discrete colony. The colonies that developed were then Gram-stained. The discrete colonies produced from previous subculture was picked with the aid of a sterile wire-loop and inoculated onto 10ml of peptone water and incubated for 24hrs at $37^0$C before being stored properly in the refrigerator. This served as the stock culture. Pure isolates were characterized according to the procedure of John et al. (1994).

### 2.3. IN VITRO SCREENING OF SOIL BACTERIA FOR PLANT GROWTH PROMOTION ACTIVITIES

The isolated bacterial strains were screened in vitro to know whether the organism has phytohormes which stimulates growth and suppress the growth of deleterious microorganisms. Soil bacteria were screened for the production of indole acetic acid, phosphate solubilization, siderophore production.

#### 2.3.1. Assay for IAA production

The production of indole acetic acid (IAA) by selected 10 strains of fluorescent pseudomonads and the effect of L-tryptophan on IAA production was assayed by using Salkowski method (Glickman and Dessaux, 1995). The bacteria were inoculated in to the nutrient broth containing L-tryptophan concentrations of 0, 50, 100, 200, 300, 400 and 500 mg/l. After 48 h of growth, the bacterial culture was centrifuged and 1 ml of supernatant was mixed with 4 ml of Salkowski's reagent. The reaction mixture was incubated at room temperature for 20 mins and then light absorbance was measured immediately at 535 nm (Patten and Glick, 2002). The amount of IAA produced was calculated using the standard curve prepared with known concentration of IAA.

#### 2.3.2. Assay for siderophore production

Production of siderophore was determined by Chromazurol Sulphonate (CAS) agar method (Alexander and Zuberer, 1991). Briefly, the bacterial inoculum was spotted into the center of a CAS agar plate. After incubation at 28°C for 5 days, siderophore production was assayed by the change in the colour of the medium from blue to orange.

#### 2.3.3. Phosphate solubilization and estimation of soluble phosphate

To determine the solubilization of phosphate, the bacterial strains were streaked into Pikovskaya agar medium (Pikovskaya, 1948) and incubated at 28°C for 3 days. After 3 days, the colonies showing the clear zones around them were considered as positive. For the quantitative estimation of soluble phosphates, the bacterial strains were

inoculated into 50 ml of pikovskaya (PVK) medium and incubated at 28°C with 160 rpm on rotary shaker. At different time intervals (1, 3, 5, 7 and 10 days) the culture broth samples were drawn and used for the estimation of soluble phosphate and then checking the pH of the culture medium. After centrifuging of the medium at 10000g for 15 min, 1 ml of the supernatant was taken and mixed with 3 ml of distilled water and 1 ml of molybdatevanadate ammonium. After 20 min of incubation, the absorbance at 470 nm was measured. Phosphate solubility was determined using the standard curve of KH2PO4 (Jeon et al., 2003).

### 2.4. PREPARATION OF INOCULUM FOR FIELD INOCULATION

The test organisms from the stock culture were resuscitated by sub culturing into an enrichment media and incubated for 24hrs at $37^0$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 $37^0$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 five sterile planting pots. The planting pots were labeled appropriately and watered carefully awaiting the application of seeds.

### 2.6. SEED GERMINATION TEST

Seeds of tomato plant *(Piper nigra)* 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 45 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 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 (Table 1).

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

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

On the other hand only *Azotobacter* sp induced the siderophore production. In related study, Datta et al. (2011) found that the majority of the rhizospheric isolates regularly produced IAA and siderophores and solubilized tricalcium phosphate. Production of IAA and soluble phosphate are the most common mechanisms of action implicated in PGPR and indeed microbes demonstrating these attributes are widespread in rhizosphere (Datta et al., 2011). Inoculation of phosphate solubilizing bacteria (PSB) to crop plants improves solubilization of fixed soil P and applied phosphates ultimately resulting in improved crop growth and higher yields (Pandey *et al.*, 2006; Vikram, 2007). The favorable effect of PSB on yield of sorghum can be attributed to better growth and yield attributing characters of sorghum plants in the presence of improved available P status of soil and also due to production of growth hormones like IAA and GA by PSB strains used in the study (Vikram *et al*., 2007). In a study by Park et al. (2005), all the free-living nitrogen fixing bacteria isolated from rhizosphere of seven different plants namely sesame, maize, wheat, soybean, lettuce, pepper and rice grown in Chungbuk Province, Korea produced indole-3-acetic acid (IAA). The isolate PM-24 (*Bacillus fusiformis*) exhibiting highest nitrogenase activity and IAA production has a promising potential for developing as a plant growth promoting rhizobacteria (Park et al., 2005).

In this study, inoculation of PGPR strains increased all parameters determined in field experiment. The effects of PGPRs on the growth of crops such as wheat (Salanture *et al*., 2006), maize (Egamberdiyeva, 2007), soybean and sugar beet (Cakmakc *et al*., 2006) were explained by $N_2$ fixation ability, phosphate solubilizing capacity and phytohormons production (Nezarat and Gholami, 2009). Yasari and Patwardhan (2007) reported that application of *Azotobacter* and *Azospirillum* strains increased canola yield, pod per plant, number of branches and weight of grains. Siderophores provide a competitive advantage to producer organism over fungal pathogens for the absorption of available iron (Suresh et al., 2010). The role of siderophores in the control of diseases has been well documented by Baker and colleagues (Suresh et al., 2010). Ahamad et al. (2005) reported that in their study that 11 isolates of pseudomonads from different crop plants produced IAA. Similarly, Karnwal (2009) also reported the varying amounts of IAA production in their study. Bhromsiri and Bhromsiri (2010) selected 25 promising isolates that have potential to be PGPR on the basis of both high nitrogenase and/or IAA production activities. de Vasconcellos et al. (2010) reported that indole-acetic acid (IAA) was produced by respective strains tested and only 2% of the 103 strains presented some phosphate-solubilizing ability. Their results demonstrate the biotechnological potential of these microorganisms.

### 3.2 MEASUREMENT OF GROWTH PARAMETERS

#### 3.2.1. Plant height

The PGPR isolates significantly affected the height of *Piper nigra* plants. Results reveal that the height increased in PGPR treated plants over uninoculated control. From Table 2, it showed that the control (A) measured 3.0cm on day 5 and increased to 6.2cm 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 10.0cm on day 5 and increased to 18.0cm on day 30DAP and it has the highest measurement. Table 2 shows plant height (cm) of *Piper nigra* recorded 5-30 days after planting (DAP). Similar results have been reported by some authors elsewhere (Bashan and Hulguin, 2004; Cakmake et al., 2006; Sharifi et al., 2011). They reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as plant height. This study revealed that seed inoculation with all bacteria resulted in an increased plant height and leaf area (Table 2). Similar increases in plant height and leaf area were observed in different crops inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains (Shaukat *et al*., 2006a, b; Suresh et al., 2010; Sharifi et al., 2011). Burd *et al*. (2000) reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens. Rokhzadi et al. (2008) reported that seed priming with plant growth promoting rhizobacteria increased dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field conditions. Remarkable increase in growth characteristics was recorded in plants with combined inoculation under field conditions was also reported by Datta et al. (2011). Their (Datta et al., 2011) results clearly demonstrate the rhizocompetence and plant growth enhancing efficacy of these strains. It can be surmised that the isolated strains have strong potential to be successful biofertilizers and bioenhacers (Datta et al., 2011).

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

| TREATMENT | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|
| A (Control)-Garden soil without biofertilizer | 3.0 | 4.0 | 4.2 | 4.8 | 5.7 | 6.2 |
| B-Garden soil + Biofertilizer (*Azotobacter*) | 3.6 | 5.2 | 5.5 | 6.0 | 6.7 | 6.8 |
| C-Garden soil + Biofertilizer (*Nitrobacter*) | 3.5 | 4.7 | 4.8 | 5.1 | 5.4 | 7.2 |
| D-Garden soil + Biofertilizer (*Nitrosomonas*) | 8.5 | 9.0 | 10.0 | 10.5 | 11.5 | 12.5 |
| E-Garden soil + Biofertilizer (*Azotobacter*, *Nitrobacter*, *Nitrosomonas*) | 10.0 | 12.0 | 13.0 | 14.0 | 16.0 | 18.0 |

**3.2.2. Stem width**

The PGPR isolates significantly affected the stem width of *Piper nigra* 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 0.3-0.8cm on 5-30 days while the combination of the three isolates had the highest measurement ranging from 0.6-1.2cm on 5-30 days as in plant height. Table 3 shows stem width (cm) of *Piper nigra* recorded 5-30 days after planting (DAP).

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

| TREATMENT | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|
| A (Control)-Garden soil without biofertilizer | 0.3 | 0.3 | 0.5 | 0.6 | 0.7 | 0.8 |
| B-Garden soil + Biofertilizer (*Azotobacter* sp) | 0.4 | 0.4 | 0.5 | 0.6 | 0.6 | 0.6 |
| C-Garden soil + Biofertilizer (*Nitrobacter* sp) | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 |
| D-Garden soil + Biofertilizer (*Nitrosomonas* sp) | 0.5 | 0.52 | 0.6 | 0.5 | 0.8 | 1.0 |
| E-Garden soil + Biofertilizer (*Azotobacter* sp, *Nitrobacter* sp, *Nitrosomonas* sp) | 0.6 | 0.7 | 0.8 | 0.8 | 1.0 | 1.2 |

Zaidi and Khan (2005) have suggested that seed priming with PGPR increased dry matter accumulation and grain yield of wheat. Murty and Similar increases in plant height due to inoculation of phosphate synthesizing bacteria (PSB) have been reported in soybean, opium poppy, gram, canola, maize (Hameeda *et al*., 2006a; Vikram, 2007). The increased plant height could be a result of increased cell elongation and multiplication due to enhanced nutrient uptake by plants following inoculation with phosphate synthesizing bacteria (PSB). It can also be attributed to the production of plant growth promoting substances in the vicinity of roots by phosphate synthesizing bacteria (PSB). All efficient strains of PSB used in the study by Vikram (2007) were able to produce both IAA and GA though in different quantities. Production of plant growth promoting substances by PSB contributed to their stimulatory effect on plant growth (Chakraborty *et al*., 2006; Hameeda *et al*., 2006b; Vikram, 2007). It is well established that IAA and GA have a role in shoot and root elongation as well as in enhancing plant growth (Vikram, 2007).

**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 6.0cm, B (treated with *Azotobacter* sp) measured 8.2cm, C (treated with *Nitrobacter* sp) measured 7.3cm, D (treated with *Nitrosomonas* sp) measured 9.4cm, and E (combination of *Azotobater* sp), *Nitrobacter* sp and *Nitrosomonas* sp) measured 10.0cm. From these values, the control had the lowest measurement 6.0cm while E which was treated with the three isolates had the highest measurement.

Table 4 shows the Internode Length (cm) of tomato (*Piper nigra)* 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. Increases of in root length of *Piper nigra* plants was noticed due to inoculation with PGPR over control and are comparable with results of Vikram (2007). Similarly, promotion in growth parameters of various crop plants in response to inoculation with PGPR were reported by other workers (Kozdroja et al., 2004; Shaharoona et al., 2006; Gravel et al., 2007). In a study by Akbari et al. (2007), the roots of wheat seedling responded positively to the several bacteria inoculations by an increase in root length, dry weight and by the lateral root hairs.

**Table 4: Root Length (cm) of *Piper nigra Recorded 30 Days after Planting (Dap)***

| TREATMENT | 30 DAP |
|---|---|
| A (Control)-Garden soil without biofertilizer | 6.0 |
| B-Garden soil + Biofertilizer (*Azotobacter* sp) | 8.2 |
| C-Garden soil + Biofertilizer (*Nitrobacter* sp) | 7.3 |
| D-Garden soil + Biofertilizer (*Nitrosomonas* sp) | 9.4 |
| E-Garden soil + Biofertilizer (*Azotobacter* sp, *Nitrobacter* sp, *Nitrosomonas* sp) | 10.0 |

## 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 previous studies by some authors. Naserirad et al. (2011) showed that double-inoculation of *Azotobacter* and *Azospirillum* had the highest plant height and stem diameter when compared with other treatments. Dastager et al. (2011) in a related study, reported that seed inoculation with the strain NII-0943, resulted in significantly higher root initiation in black pepper cuttings grown under pots.

Some other researchers have shown positive effects (Javaid, 2006, 2009; Khaliq *et al.,* 2006; Javaid et al., 2008; Javaid and Mahmood, 2010; Javaid and Bajwa, 2010; Javaid and Shah, 2010; Javaid, 2011) while others reported negative or no effects (Formowitz *et al.,* 2007; Daiss *et al.,* 2008). Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported by Biswas *et al*. (2000), Asghar *et al*. (2002) and Bashan *et al*. (2004). *Azospirillum*, *Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth (Shaukat *et al*., 2006a,b). Bashan et al. (2009) reported that inoculation with growth-promoting microorganisms induced significant effects on the leaf gas exchange of these trees, measured as transpiration and diffusive resistance, when these trees were cultivated without water restrictions. Results from the study Bahrani et al. (2010) indicated that grain yield and yield components of wheat have been affected significantly by the inoculation with Azotobacter and Mycorrhiza.

It has been shown that *Azospirillum* and *Pseudomonas* had the potential for agricultural exploitation and could use as natural fertilizers (Cakmakc *et al*., 2006). It has also been shown that inoculation of plants with *Azospirillum* could resulted in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals (Bashan *et al*., 2004). Similar improvement of seed germination parameters by rhizobacteria has been reported in other plants such as pearl millet (Niranjan *et al*., 2003, 2004), maize (Egamberdiyeva, 2007), sugar beet (Cakmakc *et al*., 2006), and wheat and sunflower (Salanture *et al*., 2006; Shaukat *et al*., 2006a, b), where it was found that some PGPR induced increases in seed emergence, in some cases achieving increases up to 100% greater than controls (Nezarat and Gholami, 2009). These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as a-amylase, which have brought an increase in availability of starch assimilation. Beside, significant increase in seedling vigor would have occurred by better synthesis of auxins (Bharathi *et al*., 2004). Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR were reported by Kozdroja *et al*. (2004), Shaharoona *et al*. (2006) and Gravel *et al*. (2007). Javaid (2011) in their study reported that the co-inoculation of biopower and effective microorganisms (EM) evidently improved root and shoot growth in farmyard manure amended soil.

This study confirms the earlier studies. It revealed that under *in vitro* conditions, seed treatment with PGPR strains improved seed germination, seedling vigor, seedling emergence and seedling stand over the control. Plant growth promoting effects of PGPR strains in different crops were clearly demonstrated (El-Hawary *et al*., 2002; Wu *et al*., 2005). Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease (Lugtenberg *et al*., 2002). This is in line with the findings of Nezarat and Gholami (2009).

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 *Piper nigra* 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 *Piper nigra*. Our results suggested that PGPR are able to enhance the production of IAA, solubilization of phosphorus, and siderophore production, thereby improving growth of *Piper nigra* plant. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable *Piper nigra* cultivation in Nigeria and other developing countries.

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