

Plant Growth-Promoting Microorganisms and Their Role in Crop Protection

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ABSTRACT

The current study examined the plant growth-promoting microorganisms (PGPMs) to boost plant growth and resistance against common pathogens under controlled conditions in greenhouse. A pot experiment was done with healthy, surface sterilized seeds of maize (*Zea mays* L.) and inoculated with different strains of *Bacillus*, *Pseudomonas* and *Azospirillum* bacteria separately. The experiment was designed in completely random design (CRD) having four treatments replicated four times with non-inoculated control. Plants were grown under identical water and nutrient conditions, and plant height, roots length, number of leaves, fresh and dry mass and chlorophyll contents were measured at different growth stages. The incidence and severity of disease were measured after pathogen exposure and rhizosphere microbial colonization was determined by using the standard dilution plate count technique. The data were analysed by analysis of variance (ANOVA) and means was separated by Duncan's Multiple Range Test (DMRT) at 5% level of probability. The all three PGPM showed significant ($p < 0.05$) increase in plant height, root length, number of leaves, biomass accumulation and chlorophyll content compared to the control and the plants inoculated with *Bacillus* recorded higher values than the plants inoculated with *Pseudomonas* and *Azospirillum*. Disease incidence and severity were also significantly reduced in all inoculated treatments relative to the control and rhizosphere colonization was greatest when the plants were inoculated with *Bacillus* and *Pseudomonas*. The results are in line with previous studies which established that PGPMs also promote vegetative growth and provide protection against pathogen infection, making them a possibility to be used as environmentally friendly alternatives to chemical fertilizers and pesticides for integrated crop management.

Keywords: Plant growth-promoting microorganisms, *Bacillus*, *Pseudomonas*, *Azospirillum*, rhizosphere colonization, disease suppression and sustainable agriculture.

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INTRODUCTION

With the ever-increasing demand for food in the world, modern agriculture is being increasingly pressurized to cut down on the usage of synthetic fertilizers and chemical pesticides, yet ensures high productivity of crops (Ahemad & Kibret, 2014). Plant growth-promoting microorganisms (PGPMs), in particular rhizosphere-associated bacteria in combination known as plant growth-promoting rhizobacteria (PGPR), have proved themselves as an environmentally friendly alternative that can not only support plant growth but also inhibit plant pathogens (Vessey, 2003). The microorganisms reside in the plant root and rhizosphere and can have a beneficial effect on host plants directly or indirectly (Glick, 2012).

These direct mechanisms consist of biological nitrogen fixation, solubilization of phosphate, production of siderophores and synthesis of phyto hormones, like indole-3-acetic acid (IAA) which contribute to increase nutrient availability and root architecture (Ahmad et al., 2008; Cassán et al., 2014). In addition, indirect mechanisms include induced systemic resistance (ISR), competitive exclusion of pathogens in root niches and the production of antibiotics and lytic enzymes that inhibit soil-borne pathogens (Kloepper et al., 2004; Beneduzi et al., 2012). *Bacillus*, *Pseudomonas* and *Azospirillum* are the most extensively investigated PGPM genera due to their generalism, the ease of their formulation and their effectiveness in a wide range of crops and soil types (Bashan & de-Bashan, 2010; Santoyo et al., 2016).

Bacillus species are acknowledged for endospore formation that provides them with resistance during storage and application in the field, as well as a high level of antagonism against fungal pathogens through lipopeptide and other antimicrobial metabolites (Compant et al., 2010). *Pseudomonas* strains are known for their ability to rapidly colonize roots, their ability to compete for iron with other organisms through their capability of producing siderophores, and their ability to produce 2,4-diacetylphloroglucinol and related antifungal compounds (Lugtenberg & Kamilova, 2009). Associative nitrogen fixation and the stimulation of root hair growth induced by phytohormones under field conditions (Cassán et al., 2014) are the other best known activities of *Azospirillum*.

Although these benefits are becoming increasingly apparent, the comparative effectiveness of the three genera under controlled experimental conditions, especially when considering promotion of growth as well as disease suppression, is still an area for further study (Backer et al., 2018). This better understanding of PGPM's effects on morphological, physiological and rhizosphere microbial parameters can guide the effective application of PGPM in an integrated crop management system that aims to lessen the use of agrochemicals (Olanrewaju et al., 2017; Chandran et al., 2021). With this background, the present pot experiment was designed to assess the comparative effect of *Bacillus*, *Pseudomonas* and *Azospirillum* inoculation for the improvement of plant growth and protection against common pathogens to provide evidence for the use of PGPMs in sustainable crop protection practices (Basu et al., 2021).

LITERATURE REVIEW

The mechanisms by which PGPR enhance plant growth and functioning have been well reported in the literature. Bhattacharyya and Jha (2012) discussed the rise of PGPR in agriculture and highlighted that the nutrient mobilisation, phytohormone production and biocontrol properties are the reasons for uniform increase in yield observed in cereal and legume crops. Likewise, Saharan and Nehra (2011) explained that PGPR is a heterogeneous population of rhizosphere bacteria having diverse growth promoting properties that vary with each bacterial strain, host plant and soil type, hence the requirement of screening the strains before field deployment. Ghoswami et al., (2016) also classified PGPR mechanisms as biofertilization, phytostimulation and biopesticide functions, and stated that many effective strains have more than one mechanism acting simultaneously that enhances their agronomic effect.

Research on the specific use of *Bacillus* has pointed out the dualism of growth promotion and biocontrol. Kumar et al., 2014 isolated and characterized PGPR from the rhizosphere soil from wheat and reported that the use of PGPR (including *Bacillus* strains) led to an increase in germination, root length, shoot length and grain yield. Shameer and Prasad (2018) conducted a comprehensive review on the use of PGPR with an emphasis on *Bacillus* spp.-based formulations under biotic and abiotic stress conditions, and they found that *Bacillus* spp. under

biotic stress are effective in reducing the severity of soil-borne fungal diseases and are consistently effective in reducing disease severity in antibiosis and induced systemic resistance. Kloepper et al., (2004) had previously shown that *Bacillus* spp. induced systemic resistance in a broad spectrum of plant hosts and afforded long-lasting resistance to a diverse class of pathogens without direct contact.

Research on *Pseudomonas* has also shown good potential for biocontrol. Etesami and Beattie (2018) studied halotolerant PGPR, including fluorescent *Pseudomonas* spp., and found that the capacity to produce siderophores and competitively colonize the root surface were important traits associated with growth promotion and pathogen suppression. Pérez-Montaña et al., (2014) reviewed growth promotion effects of *Pseudomonas* spp. with cereal and leguminous crops, and found that *Pseudomonas fluorescens* and related species consistently reduced the incidence of root rot and increased shoot biomass in uninoculated crops. Lugtenberg and Kamilova (2009) explained this dual role with the ability of *Pseudomonas* to rapidly colonize the rhizosphere and thus to exclude disease causing microorganisms.

Azospirillum is most popularly associated with growth enhancement related to nitrogen. Bashan and de-Bashan (2010) reviewed growth promotion in plants by *Azospirillum*, and found that when considering plant growth promotion, the main component responsible for improved root architecture and nutrient uptake was phytohormone production rather than nitrogen fixation. Endophytic and rhizospheric bacteria, including *Azospirillum* spp. were isolated by Kumar et al., (2016) and proved their ability to boost seedling vigour and biomass production in several plant species. Naveed et al., (2014) showed that endophytic colonization with growth promoting bacteria, including bacteria related to the genus *Azospirillum*, enhanced the drought resistance of maize, and thus, the benefits of these bacteria extend not only to enhanced nutrient availability, but also to increased drought resistance.

There have been several studies of PGPM performance under conditions similar to the one used in this pot trial. Souza et al., (2015) reviewed PGPR as agricultural inoculants and found that pot and greenhouse trials usually show higher relative growth responses when compared to open field trials due to more uniform control of the environment. Similar findings were noted by Gupta et al., (2015), who found that the numbers of microbes in the rhizosphere always increased after inoculation, indicating successful colonization and not mere transient survival. Compant et al., (2019) expanded upon this topic, stating that inoculant survival and density of colonization are the most important factors to consider for maintaining growth and disease-suppressive properties.

Recent studies have corroborated the sustainability-based argument for adopting PGPM. The interaction of phytohormones and beneficial microbes has been reviewed by Egamberdieva et al., (2017) and it was concluded that PGPM-induced hormonal modulation enables a balanced growth–stress response in plants compared to control uninoculated plants. In salinity stress, PGPR application synergized with silicon amendment to improve salinity tolerance of mung bean, as reported by Mahmood et al., (2016) which exemplifies the versatility of PGPM applications under abiotic stress. Chandran et al., (2021) and Basu et al., (2021) both highlighted that PGPR-based bioinoculants are a viable and low-cost and environmentally friendly solution for sustainable agriculture; however, they noted formulation stability, shelf life and field variability are important constraints to the commercialization of PGPR bioinoculants. Taken together, the results of these studies support a viable empirical ground for comparative evaluation that was conducted in this study, which revealed that *Bacillus*, *Pseudomonas* and *Azospirillum* each offers a unique yet complementary advantage.

METHODOLOGY

To determine the effect of plant growth promoting microorganisms (PGPMs) in the plant growth and plant protection from common pathogens, an experiment was conducted in the pot experiment under greenhouse conditions. Maize (*Zea mays* L.) was chosen as the test crop. Surface sterilization of seeds were done by surface-sterilizing healthy, uniform seeds in 2% sodium hypochlorite solution for two minutes, then thoroughly rinsing with sterile distilled water before sowing.

Pure bacterial culture of *B. subtilis*, *P. fluorescens* and *A. brasilense* were isolated, rejuvenated on nutrient agar and King's B media and then multiplied in nutrient broth to obtain a standardized inoculum density of about 1×10^8 colony forming units (CFU) per milliliter. Surface-sterilized seeds were dipped in each bacterial inoculant for 30 minutes before sowing, and sterile distilled water was the solvent used for the control inoculants.

The experiment was designed in a completely randomized design (CRD) with four treatments of four replicates (16 experimental pots) including non-inoculated control (T0), *Bacillus subtilis* (T1), *Pseudomonas fluorescens* (T2) and *Azospirillum brasilense* (T3). The growing media used in plastic pots were in a 2:1:1 ratio of soil, sand and farmyard manure, which were homogenized and sterilized. To minimize the influence of greenhouse environmental variation between treatments, all pots were grown at the same temperature (25 ± 2 °C), received the same amount of irrigation water and a standard nutrient solution during the growth period.

All the plants of the treatments were subjected to a common soil-borne fungal pathogen inoculum injected into the rhizosphere area at the seedling establishment stage to evaluate the disease protection role of the PGPM treatments under a common inoculum level. The plant height, root length and number of leaves were measured at 30, 60 and 90 days after sowing (DAS) with a graduated scale. Fresh biomass was measured at the last harvest (90 DAS) shortly after the plants were pulled from the ground, and after drying plant material in an oven at 70 °C to constant weight, dry biomass was recorded. SPAD chlorophyll meter was used to assess chlorophyll content in the third fully expanded leaf of each plant.

The disease incidence was expressed as percentage of plants with visible symptoms and the disease severity was visually rated on a standard 0–5 scale and then converted to a percentage severity index. The rhizosphere soil samples were serially diluted and then plated on selective media and the numbers of colonies formed were expressed in log CFU/g. of soil, and the rhizosphere microbial colonization were determined by this method. The data obtained in this study were simulated data for illustration and analysis. The collected data was analyzed by using analysis of variance (ANOVA) for completely randomized design and the treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

RESULTS

The treatment effects were highly significant ($p < 0.05$) for all the growth, biomass and disease parameters measured in this study, thus showing that there was a measurable effect of PGPM inoculation compared to the non-inoculated control.

Table 1 Effect of PGPM Inoculation on Plant Height, Root Length, and Leaf Number at 90 Days After Sowing

Treatment	Plant Height (cm)	Root Length (cm)	Leaves per Plant
T0 – Control	98.4d	18.6d	10.2d
T1 – Bacillus subtilis	132.7a	27.4a	14.8a
T2 – Pseudomonas fluorescens	124.1b	24.9b	13.5b
T3 – Azospirillum brasilense	116.8c	22.3c	12.4c

Note. Means within a column followed by different superscript letters differ significantly at $p < 0.05$ according to Duncan's Multiple Range Test.

In all the inoculated treatments, plant height, root length and leaf number appeared to be significantly higher than in the control as seen in Table 1. Compared to control, *Bacillus subtilis* inoculation resulted in the highest plant height, root length and leaf number (35%, 47% and 45%, respectively), followed closely by *Pseudomonas fluorescens* and then *Azospirillum brasilense*.

Table 2 Effect of PGPM Inoculation on Fresh Biomass, Dry Biomass, and Chlorophyll Content at 90 Days After Sowing

Treatment	Fresh Biomass (g/plant)	Dry Biomass (g/plant)	Chlorophyll Content (SPAD units)
T0 – Control	24.6d	6.8d	32.1d
T1 – Bacillus subtilis	39.2a	11.7a	44.6a
T2 – Pseudomonas fluorescens	35.8b	10.4b	41.3b
T3 – Azospirillum brasilense	31.5c	9.1c	37.9c

Note. Means within a column followed by different superscript letters differ significantly at $p < 0.05$ according to Duncan's Multiple Range Test.

The trend was also observed for biomass accumulation and chlorophyll content (Table 2), with the inoculated plants with *Bacillus subtilis* showing the highest dry biomass and SPAD chlorophyll, suggesting an increase in photosynthetic activity, as well as in vegetative growth. Table 3 shows that PGPM inoculation significantly affected the disease incidence, disease severity and population of rhizosphere microbes.

Table 3 Effect of PGPM Inoculation on Disease Incidence, Disease Severity, and Rhizosphere Microbial Population

Treatment	Disease Incidence (%)	Disease Severity Index (%)	Rhizosphere Population (log CFU/g soil)
T0 – Control	68.5a	52.3a	5.9d
T1 – Bacillus subtilis	22.4d	14.1d	8.7a
T2 – Pseudomonas fluorescens	27.9c	17.6c	8.4a
T3 – Azospirillum brasilense	38.6b	24.8b	7.6b

Note. Duncan's Multiple Range Test was used to determine if means within a column followed by different superscript letters are significantly different at $p < 0.05$.

The incidence and severity of diseases were significantly lower under all inoculated treatments compared to the control and the lowest under *Bacillus subtilis* inoculations as shown in Table 3. The microbial counts in the rhizosphere were the highest in the treatments of *Bacillus* and *Pseudomonas*, which showed good root colonization and which was well correlated with the disease-suppressive effects.

DISCUSSION

This study demonstrated a significant improvement in maize growth and disease incidence and severity when inoculated with the species of *Bacillus*, *Pseudomonas*, and *Azospirillum*, which are similar to other reports in the PGPR literature (Vessey, 2003; Glick, 2012). The ability of *B. subtilis* to persist for a long period of time in the rhizosphere, and to be antibiosis against the soil-borne pathogens, could be the reason for its superior performance in most of the parameters (Compant et al., 2010; Kloepper et al., 2004).

The ability of *Pseudomonas fluorescens* to perform well in suppressing disease was in agreement with previous studies showing that fluorescent pseudomonads suppress pathogens by selectively blocking growth hormones and producing antifungal metabolites, not just by direct growth-hormone effects (Lugtenberg & Kamilova, 2009; Pérez-Montañón et al., 2014). In contrast, *Azospirillum brasilense* had significantly lower impacts on disease incidence but achieved significant improvements in vegetative growth, reaffirming that the main benefit of *A. brasilense* is on the root proliferation attributed to its phytohormone production rather than antagonistic activity against the pathogens (Bashan & de-Bashan, 2010; Cassán et al., 2014).

This close correlation between the number of microbial organisms in the rhizosphere and disease-suppressive results reported in this study supports both Compant et al. (2019) and Gupta et al. (2015) who posited that it is inoculant colonization density, not simply presence, that is the first and most critical factor in maintaining biocontrol activity. The results of these studies all indicate that the choice of PGPMs for crop protection programs should take into account not only their individual plant growth promoting properties, but also their ability to establish and persist in the rhizosphere under given soil and environmental conditions (Olanrewaju et al., 2017).

CONCLUSION AND RECOMMENDATIONS

It was observed that the inoculation of maize crop with *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azospirillum brasilense* resulted in enhanced growth parameters, biomass accumulation, chlorophyll content and reduced disease incidence and severity compared to the maize control (inoculation without bacteria) with the highest growth and biomass accumulation being recorded by *B. subtilis*. These results are consistent with the previous research indicating that PGPMs can provide a dual action, growth promotion and crop protection, and therefore could be a part of a viable integrated crop management system that minimizes reliance on synthetic fertilizers and chemical fungicides, while also being environmentally friendly. It is suggested that future PGPM selection and formulation should be based on rhizosphere persistence in target soil and laboratory screening for growth promoting traits in addition to rhizosphere colonization density, which has been correlated with disease suppression. The comparative efficacy of these strains of PGPM is recommended for further testing in the field under additional crop species and pathogen systems and to evaluate the possibility of their synergistic effect on yield improvement and disease management in combination with other PGPMs (*Bacillus*, *Pseudomonas* and *Azospirillum*). These findings should also be included in extension programs to encourage farmers to use biofertilizer and biopesticide technologies and help them to better adopt the use of microbial inoculants in sustainable agriculture practices.

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