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EVALUATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FOR THE CONTROL OF CHARCOAL ROT OF MUNG BEAN

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Abstract

This research work was performed to evaluate the role of plant growth promoting rhizobacteria (PGPR) to control charcoal rot disease of Mung bean. The PGPR, *Pseudomonas stutzeri* and *Pseudomonas putida* were used to inoculate the seeds of two varieties of Mung cv. NM-11 and cv. Chakwal prior to sowing. The growth of fungus *Macrophomina phaseolina* (Causal agent of charcoal rot) was checked in dual culture assay in the presences of *Pseudomonas stutzeri* and *Pseudomonas putida.* The soil was infested with *Macrophomina phaseolina* spores before sowing the seeds. The experiment was conducted in pots under greenhouse condition. After 12 weeks of seed germination, nutrients content, disease and growth parameters were measured. Both the PGPR inhibited the growth of *M. phaseolina* and decreases the incidence of disease (DI), disease severity index (DSI) and disease mortality in both the varieties of Mung bean under stressed condition. There was maximum (53%) reduction in incidence of disease and (53%) disease severity index and (60%) disease mortality in cv. Chakwal was due to *P. putida*. Both the PGPR significantly enhanced all the growth parameters such as length of shoot and root and their weight. PGPR application, also alleviated *M. phaseolina* induced inhibition in nutrients content of leaves. *P. putida* being more effective than *P. stutzeri* in both the varieties of Mung bean and recommended for future use as biofungicides.

Key words: Disease severity index, *M. phaseolina,* Mung bean, Pathogens, PGPR, *Pseudomonas stutzeri*, *Pseudomonas putida*.

Introduction

Vigna radiata L. Wilzeck commonly known as Mung Bean, green gram nutrient rich, short duration and warm season crop which are cultivated both in the arid and irrigated conditions (Islam *et al.*, 2012). Mung bean is cultivated in Pakistan on 146,000 hectares with 98,000 tons annual production (Khan *et al.*, 2019).

PGPR are native to plant rhizosphere and act as biocontrol agents against different diseases of plant (Bhattacharyya & Jha, 2012). PGPR enhance uptake of several mineral elements from a restricted soil micro and macro nutrient pool (Gabriela *et al.*, 2015). PGPR transform the function of roots, enhance the plant nutrition and increase the plant physiology (Vacheron *et al.*, 2013). Different strains of PGPR used in agriculture to increase plants growth and to control the pests, fungal, viral and bacterial diseases (Bhattacharyya & Jha 2012).

Charcoal rot is an important disease of Mung bean production in Pakistan and world arid and tropical regions. *M. phaseolina* causal agent of charcoal rot is a seed born and soil born fungal pathogen. fungal which belongs to family Ascomycetes and affect the plants all growth stages. Mung bean is significantly cash crop most widely cultured in arid regions of Pakistan which encounters *M. phaseolina* disease (Khan *et al.*, 2018) Different fungal pathogens including Trichoderma sp. and bacterial strains such as *Pseudomonas* sp. and *Bacillus* sp., have been used effectively as control agents for the soil and seed borne fungal pathogens such as charcoal rot and *Rhizoctonia solani* in several crop (Simonetti *et al.*, 2015). The study was aimed; to control the charcoal rot disease in Mung bean caused by fungal pathogen by using PGPR as biocontrol agent.

Material and Methods

The experiment was placed under greenhouse condition in plastic pots in NARC (National Agricultural Research Center), Islamabad, Pakistan. The fungal strains (*Macrophomina phaseolina*) and seeds of two Mung bean varieties, Mung cv. NM-11 and cv. Chakwal, were used.

Fungal inocula preparation and multiplication: Inocula was prepared in potato dextrose broth. A 4 mm disk of 5 d old fungus culture was placed in flask containing potato dextrose broth, incubated in dark at 30°C for 15 d until a thick sclerotial mat developed on potato dextrose broth. Fungus were cultured Potatoes Dextrose Agar (PDA) plates. The inoculum thus produced was used in experiment.

Dual culture assay: Antagonism of bacterial strains against fungal pathogen was checked. Fungal mycelial disc 4 mm placed in the Petri dish that contained Potato dextrose agar (Dos *et al*., 1984). The percentage growth inhibition (PGI) of the fungal pathogens was observed by using the following formula (Sivan *et al.*, 1987).

$$PGI = [1 - (\text{Growth of fungus} / \text{control growth})] \times 100$$

Sterilization of seeds and bacterial inocula preparation: The seeds of both the varieties of Mung cv. NM-11 and cv. Chakwal were sterilized in 95% ethanol for 4-5 min. Subsequently washed 4-5 times with autoclaved distilled water. *Pseudomonas stutzeri* (KX574858) and *Pseudomonas putida* (KX574857) of PGPR were used for inoculation of seeds. LB broth media was inoculated with 24 hrs. Old cultures of PGPR and incubated in the shaking incubator. Before sowing the seeds, soil was infested with *Macrophomina phaseolina* spores. Sterilized seeds were soaked in the bacterial inocula for three to four hrs. and seeds

were sown in pots and filled with autoclaved soil and seeds were then allowed to grow under greenhouse conditions.

Nutrient analysis: Nutrient's analysis in plant leaves were measured by using the method of (Wolf, 1982). Leaf samples of both the varieties were dry and crushed, these sample were digested at 60°C in conc. sulfuric acid and nitric acid. The element conc. was expressed as mg / g Dw.

$$\text{Nutrient conc.} = \frac{\text{Reading of AAS} \times \text{Slope} \times \text{Dilution factor}}{W}$$

Disease assessment: The plants were observed visually at regular time intervals for the appearance of charcoal rot disease. After 27th day of inoculations morphological changes appeared due to disease. Therefore, disease incidence % and mortality % were calculated by using the method of Cohen *et al.*, (2000).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of plants}} \times 100$$

Disease severity index (DSI) estimated by using the formula of Bhattacharya *et al.*, (1985).

$$\text{DSI (\%)} = 0\,(H^n) + 1\,(S^n) + 3\,(D^n) / \text{total number of plants estimated}$$

Statistical analysis

The results were statistically observed. Least significant differences (LSD), the analysis of variance among the treatments were carried out according to Statistix 8.1 version software.

Results

Percentage growth inhibition: Fig. 1 revealed that both the PGPR showed incompatible action against *M. phaseolina* fungal growth in dual culture assay. *Invitro* dual culture assay revealed that as compared to control *M. phaseolina* showing 58.13 percentage growth inhibition with opposed *Pseudomonas stutzeri* whereas the percentage growth inhibition of *M. phaseolina* was higher due to *Pseudomonas putida* showing 49.66 percentage growth inhibition.

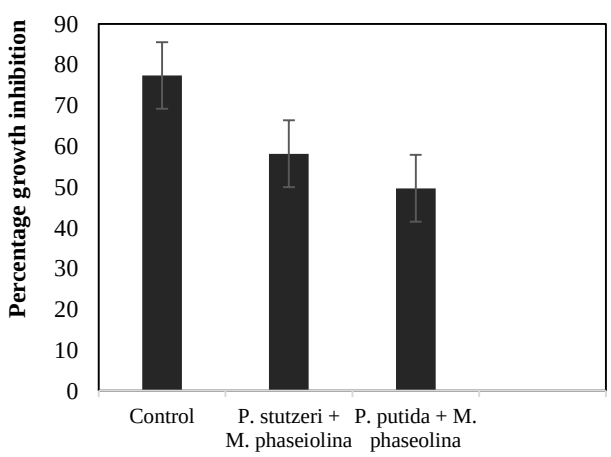

Fig. 1. Percentage growth inhibition (PGI) of *M. phaseolina* against different bacterial isolates.

Effect of PGPR and *M. phaseolina* on macronutrients (mg/g Dw): There was significant effect of *M. phaseolina* infection on the nutrient contents of *Vigna radiata* leaves in both Mung cv. NM-11 and cv. Chakwal varieties, however, all other treatments showed significant increases over untreated and infected control (Table 1). Both the PGPR were more effective under uninfected condition, *P. putida* performed better both in uninfected and infected condition. The maximum increase N (79%), K (87%) and P (66%) respectively was due to *P. putida*. The least increase N (66%), K (72%) and P (50%) respectively was due to *P. stutzeri*. Similar trend was followed by cv. Chakwal, the maximum increase N (78%), K (89%) and P (76%) respectively was due to *P. putida*. The least increase N (66%), K (78%) and P (58%) respectively was due to *P. stutzeri*. The P content was significantly decreased by 30% due to *M. phaseolina* infection in both the varieties. But both the PGPR alleviated the inhibitory effects of *M. phaseolina* infection and also increased the nutrient content over untreated control and infected plants, the maximum increase N (21%), K (18%) and P (29%) in Mung cv. NM-11 and N (20%), K (23%) and P (36%) in cv. Chakwal respectively was due to *P. putida*.

PGPR and *M. phaseolina* effect on growth parameters of Mung bean: The effects of *M. phaseolina* infection was significant on growth (length and weight) of *Vigna radiata* shoot and root in both Mung cv. NM-11 and cv. Chakwal varieties, however, all other treatments showed significant increases over untreated and infected control (Table 2). Both the PGPR were more effective under uninfected condition, *P. putida* performed better both in uninfected and infected condition. The length of root and shoot showed maximum increase (67% and 79%) respectively and the maximum increase in root and shoot weight (58% and 206%) respectively was due to *P. putida* in c.v Chakwal. The shoot and root length were significantly decreased by 19% and 4% respectively, due to *M. phaseolina* infection in both the varieties. But both the PGPR alleviated the inhibitory effects of *M. phaseolina* infection and also increased the length of root and shoot and there weight over untreated control and infected plants, The length of root and shoot showed maximum increase (19% and 36%) respectively and maximum increase in root and shoot weight (42% and 121%) respectively was due to *P. putida* in c.v Chakwal.

Disease assessment: Soil infested with *M. phaseolina* showed maximum disease incidence (66% and 63%), disease severity index (83% and 70%) and disease mortality (75% and 67%) in both Mung cv. NM-11 and cv. Chakwal respectively (Table 3). Both the PGPR reduced the *M. phaseolina* induced occurrence of disease under stressed condition, *P. putida* showed maximum reduction, significantly reduced the incidence of disease (43% and 53%), severity index of disease (57% and 53%) and disease mortality (67% and 62%) and in both Mung cv. NM-11 and cv. Chakwal, respectively. However, the untreated control has incidence of disease (35% and 35%), severity index of disease (52% and 47%) and disease mortality (56% and 62%) in both Mung cv. NM-11 and cv. Chakwal, respectively. Application of *P. stutzeri* and *P. putida* under unstressed condition both the PGPR were effective in both the varieties to decrease the incidence of disease, disease severity index and mortality, cv. Chakwal was more tolerant than Mung cv. NM-11 showed (68%, 69% and 68%) decrease in incidence of disease, severity index of disease and disease mortality respectively was due to *P. putida*.

Table 1. Effect of PGPR and *M. phaseolina* on macronutrients (mg/g Dw) in mung bean leaves.

Treatments	Mung cv. NM-11			cv. Chakwal		
	N (mg/g Dw	K (mg/g Dw)	P (mg/g Dw)	N (mg/g Dw)	K (mg/g Dw)	P (mg/g Dw)
Control	$40^{de} \pm 0.20$	$15.37^{e} \pm 0.57$	$11.16^{d} \pm 0.26$	$40.58^{d} \pm 0.38$	$14.96^{e} \pm 0.98$	$11.55^{d} \pm 0.45$
M. phaseiolena	$36.66^{de} \pm 0.22$	$11.83^{f} \pm 0.98$	$7.78^{e} \pm 0.415$	$36.58^{d} \pm 0.30$	$11.91^{f} \pm 1.06$	$8.12^{e} \pm 0.14$
P. stutzeri	$66.33^{b} \pm 2.68$	$26.43^{b} \pm 0.47$	$17.43^{b} \pm 0.47$	$67.33^{b} \pm 2.11$	$26.66^{ab} \pm 0.72$	$18.21^{ab} \pm 0.77$
P. putida	$71.66^{a} \pm 2.02$	$27.76^{ab} \pm 0.62$	$19^{ab} \pm 0.88$	$72.33^{a} \pm 1.76$	$28.33^{a} \pm 0.66$	$20.31^{a} \pm 0.94$
P. stutzeri + M. phaseiolena	$47.28^{c} \pm 1.04$	$17.33^{cd} \pm 0.44$	$14^{c} \pm 0.98$	$47.01^{c} \pm 0.87$	$16.50^{de} \pm 0.86$	$14.53^{c} \pm 1.00$
P. putida + M. phaseiolena	$48.53^{c} \pm 0.34$	$18.21^{c} \pm 0.16$	$15^{c} \pm 1.81$	$48.91^{c} \pm 0.58$	$18.33^{cd} \pm 0.66$	$15.66^{c} \pm 0.88$

Mean values followed by various letters in the same column are significantly different according to LSD $p<0.05$. The ± represent standard errors of mean (n=3).Control: untreated control seeds; *M. Phaseolina*: untreated seeds grown in infested soil with *M. phaseolina*; *P. stutzeri*: inoculation of seeds with *Pseudomonas stutzeri*; *P. putida* : inoculation of seeds with *Pseudomonas putida*; *P. stutzeri + M. phaseolina* : soil infested with *M. phaseolina* spores and inoculation of seeds with *Pseudomonas stutzeri*; *P. putida + M. phaseolina* : soil infested with *M. phaseolina* spores and inoculation of seeds with *Pseudomonas putida*

Table 2. Effect of PGPR and *M. phaseolina* on growth of mung bean plants in green house experiments.

Treatments	Mung cv. NM-11				cv. Chakwal			
	Shoot length (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Shoot length (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)
Control	$11.56^{d} \pm 0.29$	$0.28^{d} \pm 0.00$	$7.86^{d} \pm 0.09$	$0.18^{b} \pm 0.02$	$11.89^{c} \pm 0.10$	$0.29^{d} \pm 0.00$	$8.7^{c} \pm 0.25$	$0.19^{c} \pm 0.02$
M. phaseiolena	$9.42^{e} \pm 0.24$	$0.27^{d} \pm 0.01$	$7.55^{d} \pm 0.09$	$0.13^{c} \pm 0.01$	9.80d ± 0.15	$0.27^{d} \pm 0.00$	$8.5^{c} \pm 0.30$	$0.20^{c} \pm 0.00$
P. stutzeri	$18.50^{b} \pm 0.28$	$0.76^{b} \pm 0.00$	$12.18^{b} \pm 1.10$	$0.27^{a} \pm 0.00$	$19.77^{a} \pm 0.42$	$0.79^{b} \pm 0.00$	$13.80^{a} \pm 0.13$	$0.28^{ab} \pm 0.00$
P. putida	$20.16^{a} \pm 0.60$	$0.87^{a} \pm 0.01$	$13.54^{a} \pm 0.02$	$0.28^{a} \pm 0.00$	$21.24^{a} \pm 0.66$	$0.89^{a} \pm 0.01$	$14.54^{a} \pm 0.27$	$0.30^{a} \pm 0.00$
P. stutzeri + M. phaseiolena	$14^{a} \pm 0.28$	$0.58^{c} \pm 0.00$	$7.91^{d} \pm 0.19$	$0.24^{a} \pm 0.00$	$15^{b} \pm 0.76$	$0.61^{c} \pm 0.02$	$8.81^{c} \pm 0.13$	$0.26^{b} \pm 0.00$
P. putida + M. phaseiolena	$14.62^{c} \pm 0.36$	$0.62^{c} \pm 0.02$	$9.43^{c} \pm 0.27$	$0.25^{a} \pm 0.00$	$16.22^{b} \pm 0.23$	$0.64^{c} \pm 0.02$	$10.33^{c} \pm 0.25$	$0.27^{ab} \pm 0.00$

Means values followed by various letters in the same column are significantly different according to LSD $p< 0.05$. The ± represent standard errors of mean (n=3)

Table 3. Effect of PGPR and *M. phaseolina* on disease incidence (DI), disease severity index (DSI) and disease mortality in mung bean plants.

Treatments	Mung cv. NM-11			cv. Chakwal		
	Disease incidence (%)	Disease severity index (%)	Disease mortality (%)	Disease incidence (%)	Disease severity index (%)	Disease mortality (%)
Control	$43.33^{b} \pm 3.33$	$39.33^{b} \pm 5.60$	$33.33^{bc} \pm 8.33$	$41.11^{b} \pm 4.84$	$37.27^{bc} \pm 4.53$	$25.00^{b} \pm 0.00$
M. phaseiolena	$66.16^{b} \pm 6.66$	$82.66^{a} \pm 11.39$	$75.00^{a} \pm 14.43$	$63.33^{a} \pm 8.81$	$70.00^{a} \pm 10.00$	$66.66^{a} \pm 16.66$
P. stutzeri	$40.00^{b} \pm 11.54$	$29.60^{b} \pm 0.94$	$8.33^{cd} \pm 8.33$	$24.44^{bc} \pm 4.44$	$30.38^{c} \pm 0.87$	$23.33^{b} \pm 1.66$
P. putida	$26.66^{b} \pm 6.66$	$28.94^{b} \pm 0.46$	$0.00^{d} \pm 0.00$	$20.00^{c} \pm 0.00$	$21.44^{c} \pm 8.25$	$21.66^{b} \pm 1.66$
P. stutzeri + M. phaseiolena	$41.00^{b} \pm 0.00$	$46.55^{b} \pm 1.06$	$41.66^{b} \pm 8.33$	$37.77^{b} \pm 2.22$	$49.10^{b} \pm 0.77$	$50.00^{a} \pm 0.00$
P. putida + M. phaseiolena	$37.67^{b} \pm 2.22$	$35.55^{b} \pm 5.63$	$25.00^{bcd} \pm 14.43$	$29.77^{bc} \pm 1.15$	$33.21^{bc} \pm 5.74$	$25.00^{b} \pm 0.00$

Means values followed by various letters in the same column are significantly different according to LSD $p< 0.05$. The ± represent standard errors of mean (n=3)

Discussion

The data obtained during the present investigation revealed that PGPR decreased the growth of *M. phaseolina* as evidenced by dual culture assay; the percentage growth inhibition was also recorded greater than 50 % of control. The observed higher decrease in the incidence of disease following inoculation with *P. putida* may be attributed to the greater percentage inhibition in *M. phaseolina* growth as recorded in dual culture assay. Significant, effectivity of both the PGPR were higher under unstressed condition demonstrating that the inoculation with PGPR under unstressed condition is also beneficial for inducing resistance against *M. phaseolina* infection. The *Pseudomonas spp* were used as biocontrol agent against different diseases of plant (Jangir *et al.*, 2018). Kaur *et al.*, (2013) demonstrated that PGPR inoculation showed reduction in growth of pathogens. Simonetti *et al.*, (2015) demonstrated that incompatible actions of different bacterial strains against *M. phaseolina* among these *Pseudomonas fluorescens* exhibited the maximum percentage (62 %) of inhibition *in vitro*. Their research is reliable with the results of our research work. Kumar *et al.*, (2007) worked on the biocontrol of charcoal rot disease of chickpeas which is caused by *M. phaseolina* by the treatment of a strong incompatible bacterial isolate. Mhlongo *et al.*, (2018) demonstrated decrease in the incidence of disease and disease severity index (DSI) following PGPR inoculation which may be attributed to the existence of antimicrobial complexes, antibiotics, siderophores several enzymes e.g., SOD, POD and CAT which induce systemic resistance in several crops. The ameliorative effects of PGPR inoculation were also evident in the infected plants of cv. Chakwal furthermore disease occurrence and severity index of disease and disease mortality showed significant decrease on PGPR inoculation as compared to Mung cv. NM-11. Thereby demonstrating maximum (53 %) reduction in incidence of disease and (53%) disease severity index and (60%) disease mortality respectively in cv. Chakwal.

During the present work plants inoculated with *Pseudomonas stutzeri* and *Pseudomonas putida* showed much higher minerals concentration (K, N, and P) in both under unstressed and stressed conditions in both the varieties of Mung bean, cv. Chakwal showed higher mineral concentration in the infected plants as compared to Mung cv. NM-11 showed significant increase in the N (20%), K (23%) and P (36%) respectively. Hashem *et al.*, (2017) described that plant inoculation with PGPR strains improved nutrients uptake such as K, N, P, Cu, Ca, Mg and Zn which resulted to increase plants growth and help the plant to adopt to stress condition in better way by regulating various metabolic pathways such as chlorophyll synthesis and antioxidant system. Nadeem *et al.*, (2014) showed that plants inoculated with PGPR strains contain higher mineral concentration particularly increased with protein and secondary metabolites production and provide defense related gene against *M. phaseolina*. Previous study showed that PGPR inoculated plants produce phytohormone and improved the uptake of P, N, P, and Cu and other nutrients which reduce growth of pathogens (Yang *et al.*, 2010).

Th greater positive effect of *Pseudomonas stutzeri* and *Pseudomonas putida* on growth parameters may be attributed to the greater potential of PGPR to reduce the disease charcoal rot caused by fungal pathogen highly showing enhancement in length of root and shoot and their weight in Mung bean. Ricci *et al.*, (2019) demonstrated that PGPR treatment improved the shoot and root fresh weight and their dry weight and plant length and their height. Freitas *et al.*, (2015) explained similar results that plant inoculated with PGPR strains improved the different growth parameters of plant. Kiani *et al.*, (2015) explained that under stress condition the growth of different plants was significantly improved with PGPR strains.

Conclusion

Remarkably, both the PGPR were more effective under unstressed condition demonstrating that the inoculation with PGPR under unstressed conditions impart tolerance to the plants to pathogen infection in much better way than untreated control plants. It is confirmed from the observations that PGPR strains, *P. stutzeri* and *P. putida* can be exploited to effectively alleviate the effect of charcoal rot in both the Mung bean varieties.

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