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COMBINED EFFECTS OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND FUNGI ON MUNG BEAN (*VIGNA RADIATA* L.)

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ABSTRACT: In the present study, screened PGPR and Fungi were influence the growth of Mung bean (*Vigna radiata*) plant in the pot. Two rhizobacteria viz. *Rhizobium* sp., *Pseudomonas putida* and three fungi *Aspergillus niger*, *Rhizopus* sp. and *Trichoderma viride* were isolated and purified. The effect of inoculation of different strains of bacteria and fungus on growth responses of *Vigna radiata* under pot condition was enumerated. The result revealed that the single and dual inoculation of these microbial strains enhances the plant growth in terms of root and shoot length and dry-biomass. The maximum increase in root length (up to 86.57%), shoot length (up to 56.91%), root dry weight (up to 94.42%), and shoot dry weight (up to 56.09%) was observed in response to dual inoculation of *Pseudomonas putida* with *Trichoderma viride* compared to uninoculated control.

INTRODUCTION: Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield [1, 2, 3]. The important traits of PGPRs include fixation of atmospheric nitrogen, solubilization of insoluble inorganic phosphates, production of plant hormones, siderophores, bacteriocins *etc.* These organisms also provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms [4].

The potential to use PGPR in integrated strategies to reduce Nitrogen and Phosphorus fertilizers offers an appealing research area for those scientists engaged in growth promotion studies in dependable of biological control.

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As with attempts to employ PGPR will be aided by clear elucidation of mechanisms of growth promotion. Significant increase in growth and yield of agronomical important crops including cereals, pulses, vegetables, oilseeds and plantation crops in response to inoculation with PGPR has been reported [2, 3, 5, 6, 7].

In the recent years, PGPR have received worldwide importance for agricultural benefits as they are the potential tools for sustainable agriculture and have shown significant increases in growth and yield of agricultural crops both under greenhouse and field conditions [8, 9, 10, 11, 12]. Besides promoting plant growth, PGPR ensure the availability of nutrients and enhance the nutrient use efficiency [2, 3, 13, 14].

Hence, an attempt was made to study the Plant Growth Promoting Rhizobacteria (PGPR) and Fungi associated with *Vigna radiata* (Mung bean) plant to know whether a combination of PGPR and Fungi would enhance growth promotion activity under pot condition. Therefore, in this study, we decided to combine Rhizobacteria and Fungi which

have been shown to enhance plant growth activity of *V. radiata* as compare to individual and control. The study of combining these two organisms is of great potential value to organic agriculture in order to avoid fertilizers and pesticides.

MATERIALS AND METHODS:

Isolation of PGPR from Mung bean rhizosphere: Isolation of PGPR isolates were made from rhizosphere soil of Mung bean grown in irrigated fields. Ten grams of rhizosphere soil were taken into a 250 ml of conical flask, and 90 ml of sterile distilled water was added to it. The flask was shaken for 10 min on a rotary shaker at 120 rpm. One milliliter of suspension was added to 10 ml vial and shaken for 2 min. Serial dilution technique was performed up to 10^{-7} dilution. An aliquot (0.1 ml) of this suspension was spread on the plates of PDA medium. Plates were incubated for 3 days at 35°C to observe the colonies of the bacteria and for 8 days at 28°C to observe the colonies of fungus [15]. Typical bacterial and fungal colonies were observed over the streak. Morphologically different colonies were selected, marked and re-streaked until pure cultures were obtained.

Identification of Isolated Bacteria: To raise pure cultures of bacterial isolates, a bacterial suspension was prepared in a tube containing sterilized water. The tube was vigorously shaken to prepare bacterial suspension. The suspension was diluted to 10-6 times with sterilized water and 0.5 ml of it was poured over Nutrient Agar plate. The petri-dishes were incubated for three hours at 160°C. After incubation, the bacterial forms grow and form characteristic colony morphology. The isolated colony was again sub-cultured to obtain a pure culture. The purified bacterial isolates were then subjected to various morphological, cultural and biochemical tests according to the methods cited in the "Hand Book of Microbiology" [16] and were identified up to generic and specific levels with the help of "Bergey's Manual of Determinative Bacteriology" [17].

Identification of Isolated Fungi: Each fungus was purified using single spore culture technique. They were examined under stereo binocular microscope and single germinating spore was picked by needle and transferred to the Potato Dextrose Agar slants. The technique of James and Natalie [18] was adopted for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of lactophenol. The mycelium was spread very well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with ×10 and ×40 objective lenses respectively. The species encountered were identified in accordance with Cheesbrough [19].

Observation: Two PGPR viz. *Rhizobium* sp. and *Pseudomonas putida* and three fungi viz. *Aspergillus niger, Rhizopus* sp. and *Trichoderma viride* were obtained from the rhizosphere soil of Mung Bean.

Sterilization and Treatment of Seeds with PGPR and Fungi: Healthy seeds of mung bean have been selected and Seeds were surface sterilized with 10% chlorox for 3 min and then washed with 95% ethanol for 3 min with constant shaking; and later washed with sterilized water. In this experiment, technique for suspension preparation is the same as used in dual culture test. Seed pelleting method – fungal spore were count using hemocytometer and spore concentration adjusting to 15×10^3 conidia/ ml 10 seeds were pelleted with 3 ml.

Spore suspension for each fungi for 30 minutes following by carboxyl methyl cellulose (0.2% w/v) for 50 second and then dried in shade, after drying the seeds were pelleted with 1 ml of bacterial suspension (1.0 OD) containing gum Arabic [20]. Sterilized seeds were soaked in broth for 2 to 4 h. In case of control uninoculated seeds were dipped only in carboxyl methyl cellulose solution.

Experimental Setup: After soaking, seeds were sown in 12 pots containing autoclaved soil. Pots were designated as Control, PGPR1, PGPR2, PGPF1, PGPF2, PGPF3, PGPR1+PGPF1, PGPR1+PGPF2, PGPR1+PGPF3, PGPR2+PGPF1, PGPR2+PGPF2, PGPR2+PGPF3. In Control pot, sterilized untreated seeds were sowed. And in remaining pots seed treated by single inoculation of Bacteria and Fungi and dual inoculation of Bacteria + Fungi were sowed into the pots according to **Table 1**.

TABLE 1: EFFECT OF SEED TREATMENT

POT	SEED TREATMENT
Control	No treatment
PGPR1	*Rhizobium* sp.
PGPR2	*Pseudomonas putida*
PGPF1	*Aspergillus niger*
PGPF2	*Rhizopus* sp.
PGPF3	*Trichoderma viride*
PGPR1+PGPF1	*Rhizobium* sp. + *Aspergillus niger*
PGPR1+PGPF2	*Rhizobium* sp. + *Rhizopus* sp.
PGPR1+PGPF3	*Rhizobium* sp. + *Trichoderma viride*
PGPR2+PGPF1	*Pseudomonas putida* + *Aspergillus niger*
PGPR2+PGPF2	*Pseudomonas putida* + *Rhizopus* sp.
PGPR2+PGPF3	*Pseudomonas putida* + *Trichoderma viride*

Harvesting of the plants and analysis: Mung bean plants were harvested after 21 days of seed sowing through separating of plants from soil. The plants were washed through dipping into a vessel. Plant height (mm plant^{-1}) and root length (mm plant^{-1}) of each plant were recorded. Dry weights of shoot and root were recorded after drying in an oven for 1 day at 70°C.

RESULT AND DISCUSSION: PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms [2, 3, 6, 21, 22]. To be an effective PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce the beneficial effects. The exact mechanism by which PGPR stimulate plant growth is not clearly known, although, several hypothesis such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved [2, 3, 22].

PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability [23, 24]. Results suggest that PGPR are able to induce the production of IAA (indole acetic acid), solubilization of phosphorus, and resistance to pathogens and pests, thereby improving growth of plants [21, 24].

Effect on *Vigna radiata* after Single inoculation of PGPR and Fungi: The increment in root and shoot length and root and shoot dry weight in response to single inoculation of rhizobacteria and fungi as compared to uninoculated control was given in **Table 2**. In this case, isolate of *Rhizopus* sp. was the most effective and caused up to 56.0% increase in root length, up to 54.17% increase in root dry weight, up to 35.13% increase in shoot length and up to 35.48% increase in shoot dry weight.

Effect on *Vigna radiata* after Dual inoculation of PGPR with Fungi: The increment in root and shoot length and root and shoot dry weight in response to dual inoculation of rhizobacteria and fungi as compared to uninoculated control was given in **Table 3**. It was found that the plant growth was successfully observed and increases in root length (up to 86.57%), root dry weight (up to 94.42%), shoot length (up to 56.91%) and shoot dry weight (up to 56.09%) was observed in response to dual inoculation of rhizobacterial strain *Pseudomonas putida* with rhizobacteria *Trichoderma viride* compared to control.

In this study, we investigated the effectiveness of PGPR isolates. Most of isolates significantly increased plant height, root length, and dry matter production of shoot and root of Mung bean plant (**Table 2, 3**). The production of phytohormones namely auxins, cytokinins, and gibberellins, is the most commonly invoked mechanism of plant growth promotion exerted by PGPR[3,24,25,26].The mechanisms of growth and nitrogen fixation promotion by PGPR are not well understood; however, a wide range of possibilities including both direct and indirect effects have been suggested[27]. Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. A recent study showed that *A. niger* act as a major phosphate solublizer[28]. Since the

Rhizobium spp., which is effective in the formation of nodules and fixation of nitrogen the inoculation of plant growth-promoting rhizobacteria (PGPR) with *Rhizobium* spp. shows significant increase in yield and yield components of common bean (*Phaseolus vulgaris* L.) [12, 29].

TABLE 2: EFFECT OF SINGLE INOCULATION OF PGPR AND FUNGI ON *VIGNA RADIATA*

POT	Shoot length (mm)	Shoot dry weight (mg)	Root length (mm)	Root dry weight (mg)
Control	185.0 ± 1.63	224.6 ± 1.24	35.0 ± 0.81	64.6 ± 0.54
Rhizobium sp.	205.0 ± 1.63	251.0 ± 2.44	45.3 ± 2.08	85.0 ± 1.63
Aspergillus niger	208.6 ± 1.24	255.3 ± 2.05	49.3 ± 1.24	90.0 ± 2.15
***Rhizopus* sp.**	**250.0 ± 2.94**	**304.3 ± 4.64**	**54.6 ± 1.7**	**99.6 ± 2.49**
Trichoderma viride	239.3 ± 1.7	290.0 ± 1.63	44.5 ± 1.7	87.6 ± 2.05
Pseudomonas putida	224.6 ± 2.05	274.6 ± 2.05	45.6 ± 1.24	86.0 ± 1.63

(Means of three replications)

TABLE 3: EFFECT OF DUAL-INOCULATION OF PGPR AND FUNGI ON *VIGNA RADIATA*

POT	Shoot length (mm)	Shoot dry weight (mg)	Root length (mm)	Root dry weight (mg)
Control	185.0 ± 1.63	224.6 ± 1.24	35.0 ± 0.81	64.6 ± 0.54
Rhizobium sp.	205.0 ± 1.63	251.0 ± 2.44	45.3 ± 2.08	85.0 ± 1.63
Aspergillus niger	208.6 ± 1.24	255.3 ± 2.05	49.3 ± 1.24	90.0 ± 2.15
***Rhizopus* sp.**	**250.0 ± 2.94**	**304.3 ± 4.64**	**54.6 ± 1.7**	**99.6 ± 2.49**
Trichoderma viride	239.3 ± 1.7	290.0 ± 1.63	44.5 ± 1.7	87.6 ± 2.05
Pseudomonas putida	224.6 ± 2.05	274.6 ± 2.05	45.6 ± 1.24	86.0 ± 1.63

(Means of three replications)

CONCLUSION: The overall results of the present study indicate that the dual-inoculation had promising positive effects on growth of Mung bean grown in pots under natural conditions. Thus, it can be concluded from study that the use of dual inoculation of PGPR traits could be the more effective and novel approach for achieving better root growth and shoot growth of Mung bean grown under natural conditions.

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