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Effects of *Pseudomonas Sp.* (PT5) and *Fusarium oxysporum Schlcht* on growth of Mung bean (*Vigna radiata* L.)

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ABSTRACT: In the present study, the effect of Pseudomonas strain designated as a PT5 and *Fusarium oxysporum Schlcht* on growth responses of *Vigna radiata* under pot condition was enumerated. An experiment was laid out with four different treatments with four replications in Completely Randomized Design (CRD) at Department of Biochemistry, B.R. Doshi School of Bioscience, Sardar Patel University, V. V. Nagar, Anand, Gujarat, India. The result revealed that the inoculation of PT5 microbial strains enhances the plant growth in terms of germination per cent, root and shoot length, fresh and dry weight and vigor index. The maximum increase in germination (100±5.65 %), root length (12±1.3 cm), shoot length (18.2±1.94 cm), fresh weight (0.66±0.42 gm), dry weight (0.12±0.03 gm) and vigor index (3020.00±101.21) were observed in response to Pseudomonas as compared to uninoculated control.

Keywords: PGPR, Potato dextrose agar medium and Germination.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield. 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.

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 independable of biological control.

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. Besides promoting plant growth, PGPR ensure the availability of nutrients and enhance the nutrient use efficiency.

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.

MATERIALS AND METHODS

Isolation and Identification of Pathogen. Naturally infected mung bean plants showing wilt symptoms were obtained from a commercial field growing in Anand district, Gujarat, India. Isolation procedures of the causal pathogen were carried out from infected roots. After washing in running tap water, surface sterilized with 1% sodium hypochlorite solution for two minutes and rinsed three times in sterilized water, then dried between sterilized filter papers. Small pieces were placed on potato dextrose agar medium (PDA) supplemented with 300 mg of streptomycin sulfate/L in Petri-dishes and incubated at 25 ± 2°C. Pure culture was obtained using single spore technique and maintained on PDA at 27°C. Isolated pathogen was confirmed as *F. oxysporum* Schlcht by their morphological character.

Inoculum Preparation and Inoculation. *F. oxysporum* sp. isolate was grown on PDA plates for two weeks. Conidia were removed from the media surface by pipetting 10 ml of sterile saline onto the plates and gently scraping the conidia off the media surface by using a sterile spatula. The conidia were then separated from mycelium fragments by pouring the suspension through a sterile piece of gauze into a sterile beaker. The concentration of conidia was adjusted to 1×10^4 conidia/ml with a hemocytometer. Inoculation was performed by dipping the surface sterilized seed into the conidial suspension in a 500 ml beaker for 5

minutes. Seeds were dipped for 5 minutes in a beaker containing a sterile water without conidia for non-inoculated control treatment. After inoculation, seeds were planted in 25-cm diameter plastic pots containing sterilized sandy soil, ten plants each.

Isolation of plant growth promoting rhizobacterium (PGPR). The bacterial antagonistic agent *Pseudomonas* sp. designated as PT5 isolate as PGRP were obtained from Lab#302B, PG department of biosciences, Sardar Patel University, Anand, Gujarat. The isolate was maintained on King's B (KB) medium and routinely sub cultured. Some slants were maintained at 4°C, and the cultures were grown at 27°C. Culture of PT5 was grown for 24 hr. at 27°C and harvested by washing the culture with sterile saline by using a sterile spatula. Cell concentration was adjusted to an optical density of 0.06 at 660 nm which was equal to 10^8 cfuml^{-1}.

Plant materials. Seeds of Mung bean (*Vigna radiata* L.) were surface sterilization with 1% Sodium hypo chloride for 2 minutes followed by three subsequent rinses in sterile distilled water. The following treatments were taken (i) Surface sterilized seeds were dipped in *F. oxysporum* (1×10^4 cells /ml) (ii) Dipped in PT5 (1×10^8 cells/ml) (iii) dipped in *F. oxysporum* + PT5 suspension for 5 minutes and (iv) Control. After that treated seeds were shade dried for 30 min. Then ten seeds were incubated in each pot with four repetition.

Observation recorded

Germination percentage was recorded every 3 days for three weeks after inoculation.

The seed germination percentage was calculated by the following method given by ISTA (Anon, 2011):

$$\text{Germination}(\%) = \frac{\text{Number of seeds germinated}}{\text{Total number seeds for tested}} \times 100$$

The length of the plant was measured in terms of root length and shoot length by using a scale and values was expressed in centimeters.

The root length and shoot length were measured from the tip of the primary root to the base of the hypocotyl in a randomly selected seedling from each replication and the mean shoot and root length was expressed.

The randomly selected seedling was taken for determining the fresh weight of the plant was recorded by using an analytical balance and expressed in milligram. While in dry weight of seedling, its dry by an oven at 72ºC for 48 hrs and their dry weight was expressed in milligrams.

The seed vigor index was calculated and expressed as a whole number. The formulas are given below:

Seedling vigor index= {Average shoot length (cm) + Average root length (cm)} × Germination (%)

RESULTS AND DISCUSSION

PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. 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.

PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability. 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.

Effect on *Vigna radiata* after inoculation of PGPR and Fungi: The results are presented in Table 1 is revealed that the cent percent germination was recorded in PT5 treated seeds followed by control without any treatment that was 91.60±4.55 per cent followed by PT5 + *F. oxysporum* treated seeds (81.33±6.21%). Relatively less germination rate (62.77±3.24%) was recorded *in F. oxysporum* infected seeds.

However, the maximum root length (12±1.3 cm), shoot length (18.2±1.94 cm), fresh weight (0.66±0.42 cm) and dry weight (0.12±0.03 cm) were observed in PT5 treated seeds as compared to other treatments. While minimum root length (6.6±1.62 cm), shoot length (14.13±2.68 cm), fresh weight (0.56±0.87 gm) and dry weight (0.09±0.04 gm) were observed in fungal pathogen treated seeds

The maximum vigor index (3020.00±101.21) was recorded in PT5 treated seed followed by control seeds (2210.30±124.32). While lowest vigor index (1301.22±87.23) was recorded in *F. oxysporum* infected seeds.

These findings are in complete agreement with many workers: Biswas *et al.* (2000); Asghar *et al.* (2004); Bashan *et al.* (2004).

Table 1: The Effect of PT5 on grown of *V. radiata* L.

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)	Vigor Index (%)
T_1. PT5	100±5.65	12±1.3	18.2±1.94	0.66±0.42	0.12±0.03	3020.00±101.21
T_2. PT5 + *F. oxysporum*	81.33±6.21	10.26±2.8	16.6±2.71	0.57±0.59	0.11±0.09	2184.52±98.78
T_3. *F. oxysporum*	62.77±3.24	6.6±1.62	14.13±2.68	0.56±0.87	0.09±0.04	1301.22±87.23
T_4. Control	91.60±4.55	7.53±1.7	16.6±2.75	0.59±0.57	0.10±0.21	2210.30±124.32
SEm ±	2.280	0.280	0.480	0.021	0.004	54.360
CD @ 5%	7.03	0.86	1.48	0.06	0.01	167.50
CV%	4.71	5.33	5.07	6.11	6.27	4.32

A B C D

Plate I: Germination assay of *V. radiata L.* (A) PT5 treated (B) control, (C) PT5 + *F. oxysporum* treated and (D) *F. oxysporum* infected

A B C D

Plate II: The effect of PT5 on shoot and root length of *V. radiata* L. (A) PT5 Treated, (B) control, (c) PT5 + *F. oxysporum* treated and (d) *F. oxysporum* infected.

CONCLUSIONS

The overall results of the present study indicate that the PT5 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 inoculation of PGPR traits could be the more effective and novel approach for achieving better germination, root growth and shoot growth of Mung bean grown under natural conditions.

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