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Plant growth promoting and biocontrol activity of *Rhizobium meliloti* against plant pathogens

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ABSTRACT

The present study was carried out with plant pathogenic microorganisms were isolated from the infected roots of *Vigna mungo* plant, collected from Thanajavur district, Tamil Nadu, India. The identified fungal colonies were confirmed as *Aspergillus flavus* and *Fusarium solani*. In this study plant growth promoting rhizobacterial strains were successfully isolated from the *Vigna mungo* rhizosphere soil. The isolated organisms were confirmed as *Rhizobium meliloti*. The isolated organisms were used for indole acetic acid production the isolated organism were produced high level. The seed germination test were analysed in agar plates. The *Vigna mungo* seeds were treated with isolated Rhizobial species and placed on agar surface the seed germination was counted 90% percentage seeds were germinated in this results. Analysis the Morphometric and Biochemical parameters, After 10 days of growth, germination percentage, root length, shoot length, fresh weight and dry weight were measured and calculated. Biochemical compound such as chlorophyll, total protein and total carbhohydrate were analysed. The biochemical contents such as chlorophyll, protein and carbohydrates content were notied in decreased amountthe in treatment T6 (*Aspergillus flavus* and *Fusarium solani*) containing pot, when compared with control treatment (without microorganisms).

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INTRODUCTION

PGPR is a bacterium from the root region (Rhizobacteria) that colonizes plant roots and stimulate plant growth. PGPR works by helping in nodule formation, development of root fibers, and growth of the root system which helps to promote plant health. In the past 10 years, Plant growth promoting rhizobacteria are bacteria that colonize plant roots, and in doing so, they promote plant growth and/or reduce disease or insect damage (Jetiyanon and Kloepper, 2002). Plant growth promoting rhizobacteria (PGPR) are naturally-occurring, free-living soil bacteria that are capable of colonizing roots and enhancing plant growth when added to seeds or roots (Kloepper and Schroth, 1978). Several mechanisms have been postulated to explain how PGPR stimulate plant growth, and these can be broadly categorized as either direct or indirect (Kloepper, 1993). When plant growth promotion by bacteria is direct, PGPR produce a metabolite or compound that is stimulatory to plants (e.g. a phytohormone such as indoleacetic acid), whereas bacteria that elicit plant growth effects indirectly do so by affecting other factors in the rhizosphere which in turn results in plant growth stimulation (e.g. PGPR suppression of indigenous, plant yield-reducing rhizosphere microorganisms).

Some bacteria and fungi prevent diseases and enhance plant growth. A beneficial free-living soil bacterium that increases plant growth are generally referred to as plant growth- promoting bacteria and are found in association with the roots of various plants (Kloepper *et al.*, 1991; Shanmugaiah *et al.*, 2009). Biological control using introduced microorganisms with the capacity to elicit induced systemic resistance (ISR) against plant diseases has been extensively studied under greenhouse and field conditions. Several studies have shown that individual strains of plant growth promoting rhizobacteria (PGPR) could elicit ISR against multiple diseases on one plant host (Hoffland *et al.*, 1996; Wei *et al.*, 1996). In this study isolation of Plant Growth Promoting Rhizobacteria (PGPR) were isolated in *Vigna mungo* rhizosphere soil. Plant growth promoting bacteria act as a biocontrol agent so the biocontrol activity of isolated bacteria against two plant pathogens.

MATERIALS AND METHODS

The present study was carried out with plant pathogenic microorganisms were isolated from the infected roots of *Vigna mungo* plant, collected from Thanajavur district, Tamil Nadu, India. 20 ml of sterilized and warmed PDA and Nutrient media were poured into sterilized Petri plates and allowed to solidify. The root sample were crushed and serially diluted, the sample were inoculated spread plate method for both medium. The plates were incubated at room temperature (28±2°C) for four days.

A drop of 95% alcohol was placed on a slide. A fragment of the culture was teased out using needle. When spread, the alcohol was allowed to evaporate. A drop of lacto phenol cotton blue was placed on it and a cover slip was applied, avoiding air bubbles. Then excess stain was removed using blotting paper and the preparation was screened using 45x objective of the light microscope. The plant pathogens were developed by sand-maize medium (sand and ground maize grains) mixed at the ratio 19:1 and sterilized in polythene bags at 121°C for 30 min. after sterilization pathogens were inoculated into the medium and incubated at 28±2°C for 10 days.

Soil samples were collected from the rhizosphere of 1 month old *Vigna mungo* plants in different areas of Thanjavur. The rhizosphere was dugout with intact root system. The samples were placed in plastic bags and stored at 4°C in the Laboratory. Isolation and Identification of *Rhizobium* from the rhizosphere Soil Sample Using YEMA Medium (Vincent, 1970; Somasegaran and Hoben, 1994), five different soil samples were serially diluted for the isolation of soil *Rhizobium* using YEMA medium. The test isolates were subjected to the following cultural, biochemical and microscopic tests in order to confirm their identity as

Rhizobium and differentiate them from an allied genus *Agrobacterium*.

The culture of Rhizobial isolates were streaked on YEMA agar plates and incubated at 10, 20, 28, 37 and 45°C. The change in growth and color was observed and recorded after 3 days of incubation.

The isolated Rhizobial strain was cultured in YEMA medium for about 3 days in order to establish better growth of bacteria. Three to six days old cultures of *Rhizobium* was examined for purity and the bacterial colonies were scraped with the help of an inoculation needle and was transferred to a 250 ml conical flask containing YEMA broth. The subculture flasks were incubated at 28°C on a rotary shaker for 2-3 days. This broth was used for the experimental work.

IAA production

A single colony of bacterial culture was grown on LB liquid medium. A loopful of the respective culture was transferred to the 100 mL of conical flask containing LB liquid medium with the help of a sterile inoculation needle. The flask was then incubated for 7 days on a rotary shaker.

The cultures in the flask showed dense milky white growth were tested for purity (Bric *et al.*, 1991).

Seed germination test

Vigna mungo seeds were collected from Annamalai Agro Products at Orathanadu. The seeds were soaked in H_2SO_4 for 5 min and washed with sterile water three times to remove the H_2SO_4.

Then seeds were treated with bacterial strain for 30 min. 10 seeds were placed on agar (2%, w/v) plates and incubated for 3 days in the dark. Finally, germination of seeds was recorded.

Effect of plant pathogens against *Rhizobium* in pot culture experiment

Vigna mungo seeds were surface sterilized with 1% sodium hypochlorite solution for 20 min and then the seeds were washed in sterile distilled water. Ten seeds per pot containing 25% sand: 75% red soil (250g pot) was sown and three replicates were maintained for each treatment (Ashrafuzzaman *et al.*, 2009).

Pathogen inoculated and un-inoculated controls were maintained in separate pots in the green house with the temperature ranging from 28 to 32°C and the plants were irrigated every two days.

The soil moisture content was maintained at about 70%. After 10 days of growth, germination percentage, root length, shoot length, fresh weight and dry weight were measured and calculated.

T1. Treatment- I (*Aspergillus flavus*)
T2. Treatment- II (*Fusarium solani*)
T3. Treatment- III (*Rhizobium meliloti*)
T4. Treatment- IV (*Rhizobium meliloti* + *Aspergillus flavus*)
T5. Treatment- V (*Rhizobium meliloti* + *Fusarium solani*)
T6. Treatment- VI (*Aspergillus flavus* + *Fusarium solani*)
T7. Treatment- VII (Control)

Analysis the morphometric and biochemical parameters

The percentage of seed germination was calculated from the each treated pot after one week from the sowing. Plants were collected from each treated pot after 10 day from the seed sowing. The length of the root and shoot was measured individually for plant and expressed in cm. The chlorophyll content of each pot culture plant was estimated by Arnon (1949). The total protein content was estimated from each treatment plant leaf by Lowery *et al.* (1951). The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar (1984).

RESULTS AND DISCUSSION

The isolated plant pathogenic fungal colonies were identified based on Lactophenol cotton blue staining

and results were compared with standard fungal identification manual. The identified fungal colonies were confirmed as *Aspergillus flavus* and *Fusarium solani*. In this study plant growth promoting rhizobacterial strains were successfully isolated from the *Vigna mungo* rhizosphere soil. The isolated *Rhizobium* was named as PGPR1. Microscopic observations were performed to investigate the same characteristics of PGPR isolates such as shape, gram reaction and motility (Table 1).

Table 1. Biochemical characteristics of rhizobial isolates

Biochemical test	Rhizobial strains
Indole test	+
Methyl red test	-
Voges Proskauer test	+
Citrate utilization test	+
Triple Sugar iron agar test	-
Starch hydrolysis test	+
Urease test	+
Macconkey agar test	+
Hydrogen sulphide production test	+
Catalase test	+
Oxidase test	-
Nitrate reduction test	+

+ Indicate Positive results; - Indicate Negative results

The isolated organisms were motile and gram negative in reaction. The isolated organisms were confirmed as *Rhizobium meliloti*. The isolated organisms were used for indole acetic acid production the isolated organism were produced high level. This organism improves the plant growth and phosphrous solubilization. The seed germination test were analysed in agar plates. The *Vigna mungo* seeds were treated with isolated Rhizobial species and placed on agar surface the seed germination was counted 90% percentage seeds were germinated in this results (Table 2).

The present study plant pathogenic fungal colonies were identified. The identified fungal colonies were confirmed as *Aspergillus flavus* and *Fusarium solani*. Fusarium fruit rot (FFR) is caused by two "races" of *Fusarium solani* f. sp. *cucurbitae*: race 1 and race 2. All cucurbits tested are susceptible, but generally only pumpkin (*Cucurbita pepo* L.) and winter squash (C. *pepo* L., *C. moschata* Duchesne, and *C. maxima* Duchesne) are affected in the field (Davis *et al.*, 2005); the disease is an economic problem for pumpkin growers on California's central coast, with at least 30% of the pumpkin fruit infected in some fields.

Table 2. Analyses the morphometric parameters

Treatment	Seed germination (%)	Root length (cm)	Shoot length (cm)
T1	70	4.0±1.24	9.0±1.82
T2	80	4.5±1.60	11.0±1.58
T3	100	7.6±1.22	16.5±1.62
T4	100	6.8±1.38	15.2±1.10
T5	100	7.0±1.22	15.8±1.00
T6	60	3.8±1.90	10.0±1.47
T7	80	6.0±1.10	14.0±1.15

T1. Treatment- I (*Aspergillus flavus*); T2. Treatment – II (*Fusarium solani*); T3. Treatment- III (*Rhizobium meliloti*); T4. Treatment- IV (*Rhizobium meliloti* + *Aspergillus flavus*); T5. Treatment- V (*Rhizobium meliloti* + *Fusarium solani*); T6. Treatment- VI (*Aspergillus flavus* + *Fusarium solani*); T7. Treatment- VII (Control). Values are expressed as Mean ± Standard deviation.

The shoot and root length, fresh weight of plants grown in pathogens with antagonist were anlysed in the results were presented in this chapter. The plant pathogen incorporated soil samples were grown low shoot length and root length at the same rhizobium inoculated pot high growth compared than control (Table 3, Fig. 1). At the same pre-treated *Rhizobium* culture incorporated seeds high growth and germinating ability and shoot length and root length were presented compared than control pot.

Table 3. Analysis biochemical parameters

Treatment	Chlorophyll (mg/g fw^{-1})			Protein (mg/g)	Carbohydrate (mg/g)
	A	B	Total		
T1	0.18	0.22	0.24	18.3	2.4
T2	0.20	0.26	0.29	16.7	2.2
T3	0.49	0.56	0.86	28.06	6.2
T4	0.43	0.50	0.80	26.70	5.8
T5	0.45	0.53	0.82	26.20	6.0
T6	0.12	0.18	0.32	15.26	1.8
T7	0.35	0.39	0.74	22.07	4.6

T1. Treatment- I (*Aspergillus flavus*); T2. Treatment- II (*Fusarium solani*); T3. Treatment- III (*Rhizobium*

meliloti); T4. Treatment – IV (*Rhizobium meliloti* + *Aspergillus flavus*); T5. Treatment – V (*Rhizobium meliloti* + *Fusarium solani*); T6. Treatment - VI (*Aspergillus flavus* + *Fusarium solani*); T7. Treatment – VII (Control).

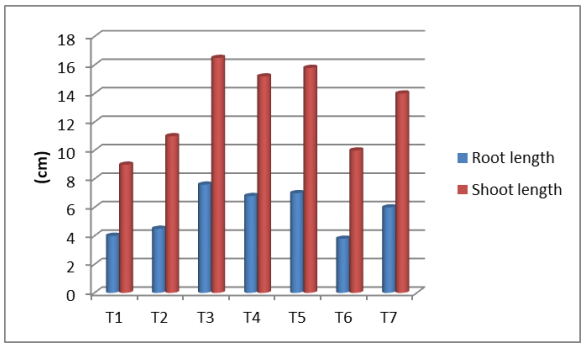

Fig. 1. Analysis of the morphometric parameters - Root length (cm) and Shoot length (cm)
T1. Treatment- I (*Aspergillus flavus*); T2. Treatment- II (*Fusarium solani*); T3. Treatment- III (*Rhizobium meliloti*); T4. Treatment- IV (*Rhizobium meliloti* + *Aspergillus flavus*); T5. Treatment- V (*Rhizobium meliloti* + *Fusarium solani*); T6. Treatment- VI (*Aspergillus flavus* + *Fusarium solani*); T7. Treatment- VII (Control).

Biochemical compound such as chlorophyll, total protein and total carbhohydrate were analyse (Table 3). The maximum level of biochemical compounds was noted in T3, T4 and T5. Among the study all the biochemical parameters such as chlorophyll, protein and carbohydrates content decreased in the T6 treatment (*Aspergillus flavus* and *Fusarium solani*) containing pot, when compared with control treatment (without microorganisms). The results reported here corroborate earlier studies and indicate a future possibility that PGPR formulations can be used to promote growth and health of crop plants. Treatments with rhizobacterial formulations significantly enhanced the growth of pearl millet plants and also reduced the percentage of downy mildew incidence.

Under salt stress, PGPR have shown positive effects in plants on such parameters as germination rate, shoots and roots (Kloepper *et al.*, 2004) another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests (Dey *et al.*, 2004). Moreover, PGPR mediate biological control indirectly by eliciting induced systemic resistance against a number of plant diseases (Jetiyanon and Kloepper, 2002). Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of induced systemic resistance in the treated plant. PGPR have also been reported in cereal crops including rice (Yanni *et al.*, 1997; Biswas *et al.*, 2000).

In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus, and production of siderophores that chelate iron and make it available to the plant root (Lalande *et al.*, 1989; Glick, 1995; Bowen and Rovira, 1999). It has also been reported that PGPR is able to solubilize inorganic and/or organic phosphates in soil (Liu *et al.*, 1992).

Effectiveness of PGPR isolates whether they could increase the seed germination rate as well as growth of seedlings. Most of isolates significantly increased plant height, root length, and dry matter production of shoot and root of *Vigno mungo* seedlings. Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Pradhan and Sukla, 2006).

CONCLUSION

In this study plant growth promoting rhizobacterial strains were successfully isolated from the *Vigna mungo* rhizosphere soil. The isolated organisms were confirmed as *Rhizobium meliloti*.

The isolated organisms were used for indole acetic acid production the isolated organism were produced high level. The *Vigna mungo* seeds were treated with isolated Rhizobial species and placed on agar surface the seed germination was counted 90% percentage seeds were

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