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Enhancement of Black Gram (*Phaseolus mungo* L.) growth by dual inoculation with *Pseudomonas fluorescens* and *Rhizobium leguminosarum*

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Abstract
There is an experimental results showed that *Pseudomonas fluorescens* and *Rhizobium leguminosarum* were able to colonise the root environment where they carried out a variety of interactive activities to enhance plant growth and soil fertility. *P. fluorescens* was isolated from rhizosphere soil and *R.leguminosarum* from root nodules. *Phaseolus mungo* L. plant (black gram) was inoculated with *P. fluorescens*, *R. leguminosarum* by using seed bacterisation inoculants and soil application methods. Seed bacterisation has been proved to be more effective than soil applications. Results of pot experiments revealed that, the co-inoculation of *P. fluorescens* and *R. leguminosarum* showed highest growth promoting effects in black gram than the individual inoculations, because of one or more growth promoting mechanisms like phytohormones, organic acids, siderophores, enzymes and exopolysaccharides produced by rhizophia in the rhizosphere soil could be responsible for evoking the growth stimulating response in the inoculated plants.

Keywords: Seed bacterisation, phytohormones, siderophores etc.

Introduction
In developing countries like India, raise in population has increased the demand for agricultural products. India population continues to grow at a rate of 1.8 to1.9% annually. Currently, India is producing two hundred million tons of food grains per annum. It will be increased to 350-375 million tons per annum over the next 2-3 decades (Daniel, 20o0)[1]. Singh, (2001) [2] reported that increasing demand for agricultural products will lead to demand for chemical fertilizers. These chemicals used in the field trials lead to many problems like pollution, host resistance and bio magnification. Gryndler, (2000) [3] has demonstrated that the soil borne microorganisms interacts with the plant roots and soil constituents at the root-soil interface. The microbial activity of the rhizosphere enhances the rooting patterns and the supply of available nutrients to plants, modifying the quality and quantity of root exudates (Linderman, 1988) [4].

Microorganisms play a major role in biogeochemical process, such as solubilization of nutrients, N_2 fixation, siderophore production, mobilization and mineralization of various plant nutrients. The beneficial effects of Rhizobia, mycorrhizae and fluorescent pseudomonads have been studied extensively in relation to plant nutrition and plant protection. However, the interactions between these organisms in the rhizosphere have received less attention. The interactions within microbial communities associated with plant roots are of considerable interest, because such interactions might either enhance or inhibit the beneficial effects of the individual species (Germida and Walley, 1996) [5]. Symbiotic nitrogen fixation is well known process exclusively driven by bacterial nitrogenase enzyme which specifically reduces atmospheric nitrogen to ammonia in the symbiotic root nodules (Leigh, 2002) [6]. The bacteria responsible belong to the genera *Rhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium* are collectively termed as Rhizobia. Sprent, (2002) [7] reported that these bacteria interact with legume roots leading to the formation of nitrogen-fixing nodules.

Azotobacter chroococcum strain AGO11 had significant effects on growth and yield of wheat in field study in some parts of Iran (Khosravi, 2009) [8]. The interaction of irrigation and

Rhizobium on root dry weight was significant, in which the highest values in treatment irrigation with a 3- day period and synchronous inoculation of *Azotobacter* and *Rhizobium* raised root dry weight amount of 26.4% Compared to control treatment [9]. Linderman, (1992) [9] showed that *Pseudomonas putida* strain R-20, a siderophore producing plant growth promoting rhizobacterium, enhanced nodulation and nitrogen fixation by *Rhizobium* on subterranean clover.

Pathogenic microorganisms affecting plant health are the major and chronic threat to food production and ecosystem stability worldwide. Over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop production. However, increasing use of chemical inputs causes several negative effects, (i.e) development of pathogen resistance to the applied agents and their non-target environmental impacts (Gerhardson, 2002) [10]. The widely recognized mechanisms of bio-control mediated by PGPB are competition for an ecological niche or a substrate production of inhibitory allelochemicals and induction of systemic resistance in host plant to a broad spectrum of pathogens (Hass and Keel, 2003) [11].

Materials and methods

1. Sample collection

Rhizosphere soil and leguminous plant root with nodules were collected from paddy field at Mantharamputhoor, Kanyakumari District, Tamil nadu.

2. Isolation of *Psuedomonas fluorescens* from rhizosphere soil

Host plants were uprooted from the field with some rhizospheric soil and brought to laboratory in polythene bags. Rhizosphere soil from the roots was removed by gentle shaking while rhizosphere soil was removed by dipping and gentle shaking in water. The diluted rhizosphere soil sample was serially diluted and plated on the King's B medium and incubated at 28 ±2 ^{0}C for 24 hours. After incubation, the fluorescent colonies were observed and characterized. Identification of selected isolates based on biochemical characterization as described in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) [12].

3. Isolation of *Rhizobium leguminosarum* from root nodules of leguminous plants

The number of nodules present on a well developed root system of leguminous plants was taken. From the roots, nodules were separated with sterilized razor blade and placed separately in Petri plates. These nodules were surface sterilized for 1 min in a 5% solution of sodium hypochlorite followed by three rinses in sterile distilled water to remove the hypochlorite. Surface disinfected nodules were crushed by means of sterilized glass rod in sterilized test tube containing sterilized distilled water. The suspension acquired was used to inoculate petriplates having autoclaved. The solidified yeast extract mannitol (YEM) (Himedia) with one pinch of congo red was used in this study for preliminary differentiation of rhizobia from non-rhizobia. After 5 days of incubation at 25°C, the *R. leguminosarum* colonies that originated from each nodule were the white color, mucoid colonies observed on the plates were isolated and identified. The selected isolates based on biochemical characterization as described in Bergey's Manual of Determinative Bacteriology characterized.

The pure colony of the isolate was later transferred to YEM broth medium with gentle shaking at 120 rpm for 5 days. By this procedure, the *R. leguminosarum* culture reached the middle or late logarithmic phase, and cell density in the culture was estimated by measuring optical density (OD) using spectrophotometer at 540 nm (OD value of 0.2). Finally the cell suspensions were diluted in 10 mM MgSO4 to 10^6CFU ml^{-1} prior to use in different experiments.

4. Pot experiments with soil application

The inocula of *Pseudomonas fluorescens* was prepared by suspending cells from a 24 h-old culture in Kings B medium in 10 mM MgSO4, to an OD560 nm of 0.3 (corresponding to 10^7 CFU ml^{-1}). Inoculum of *R.leguminosarum* was prepared similarly, but by suspending cells from a 48 h-old culture in YMA to OD560 nm of 0.2. Finally the cell suspensions were diluted in 10 mM MgSO4 to 10^6CFU ml^{-1} prior to use in different experiments. The isolates were mixed with sterile loamy soil used for soil application. Soil application was carried out by inoculated soil mixed (culture to soil ratio 1:1) with 10% sugar solution.

In the soil application study, the pulse crop *Phaseolus mungo* L. (Black gram) seeds were procured from Tamilnadu Agricultural University, Tirunelveli. The seeds were allowed to grow in four nursery pots. One was maintained as control (without inoculation) and designated as

- Second pot was inoculated with soil application of *P. fluorescens*
- Third pot was inoculated with soil application of *R.leguminosarum*
- Fourth pot was inoculated with soil application of both *P. fluorescens* and *R. leguminosarum*

5. Seed bacterization

The seeds of *Phaseolus mungo* L. (black gram) were sterilized with 2.4% sodium hypochlorite solution for 2-3 minutes and rinsed in sterile distilled water and dried overnight under sterile air stream. For inoculation, the cell suspension of each bacterial strain (*Pseudomonas fluorescens, Rhizobium leguminosarum* and for both combination of *P. fluorescens* and *R. leguminosarum*) was mixed with charcoal used for seed coating. Seed coating was carried out by inoculated charcoal mixture (seed to charcoal ratio 1:1) with 10% sugar solution. In case of control, seeds were coated by sterilized peat treated with sterilized broth plus 10% sugar solution. The surface sterilized dry seeds of *Phaseolus mungo* L. were dipped in the bacterial suspensions and dried overnight in sterile petridishes under sterile air stream. Care was taken to avoid clumping of the seeds. For co-inoculation, *R. leguminosarum* and *P. fluorescens* were mixed in 1:1 ratio for attaining all possible combinations of co-inoculants. After inoculation seeds were spread over night for drying (Iqbal *et al*., 1994) [13].

5.1. Pot experiments with seed bacterization of black gram

Four pots were selected for this experiment. One was A (control-without inoculation), B (*P.fluorescens*) inoculated seeds, C. *R. leguminosarum* bacterised seeds and D (Both inoculated seeds). The tapered cylindrical pot (16 cm height×16 cm top diameter×11 cm bottom diameter) commonly used in green house experiments, was selected for this study. This pot was approximately 2.3 litres in volume. Sand used in this experiment was collected from riverbeds of Thambaraparuni, Tirunelveli, Tamilnadu and it was washed several times, dried for 3 days at sunlight and sifted to remove debris. The sand was separated into groups based on particle sizes.

In all experiments, pots were filled to within approximately 2.5 cm of the top with a commercial sandy loam potting soil and layered with mixed soil (silt soil, red soil and clay). In the growth study experiment of black gram, 24 seeds were sown in each pot. The seeds were allowed to germinate and its germination percentage was calculated. Afterwards, plants were grown in a greenhouse subjected to natural solar radiation with the air temperature regulated between approximately 20 and 28 °C (night/day). Day length extended to 16 h by incandescent lamps over the black gram to keep the plants in vegetative development throughout the experiment. Only the required quantity of water alone was poured into each pot in order to avoid draining of excess water from the pots that can result in elimination of nutrients. The growth and growing stages of the plants were carefully monitored and the morphometric data were also taken every week (upto 4 weeks). For each data entry, three plants were randomly collected from each treatment and the observation of exo morphological characters was done. The treated groups were maintained in triplicates with a separate control. The seed germination pattern and percentage of growth were observed and noted. The calculation formula was as follows:

$$\text{Percentage germination (\%)} = \frac{\text{Final germination}}{\text{Total seeds investigated}} \times 100$$

6. Monitoring of the root colonization of black gram plant

To monitor root colonization, after 21st day of growth plants, the plants were dug out with soil particles adhering to the roots. Rhizoplane soil particles tightly attached to the roots should not removed from the roots. The roots from the seedlings cut into 1cm segments and 1gram of such pieces was dipped in 50ml of sterile distilled water. Appropriate dilutions of the suspension were poured plated on KB medium. After 48 hours at 28±2^{0}C, CFU for 1gm root segments were counted. The bacterial colonization also performed and compared with control and recorded.

7. Assay for *in vitro* antibiosis

In vitro tests for antagonism of *P.fluorescens* against common fungal pathogens like *Fusarium solani.*

7.1 Agar well cut method

In the experiment, a fungistatic activity of *P. fluorescens* against of *R. solani* has been assessed. The bacteria were cultured in the broth medium for 24 hours at 30ºC. Next, the broth was inoculated with the suspension of 10^6 cfu/ml *P. fluorescens* and incubated for the time period of 48 hours. The culture supernatant of different concentration of *P. fluorescens* culture (µl) was poured in the wells cut in Muller Hinton Agar plates and incubated overnight at 37^{0}C. After incubation, zone of inhibition was measured (mm).

7.2 Hydrogen cyanide (HCN) production

HCN production was detected on King's B medium amended with glycine. Filter paper dipped in 0.5% picric acid and 2% sodium carbonate was placed inside the lid of the sealed petriplate. HCN production was indicated by yellow to deep brown colour on the picric acid dipped filter paper.

7.3 Detection of phosphate solubilization

A loopful of 48 hours old culture of *P.fluorescens* was streaked on Pikovskaya's agar medium for the detection of phosphate solubilization. The plates were incubated at 28±2^{0}C for 6 days and if the organisms produced phosphatase enzyme, the zone of solubilization around the growth was observed.

7.3.1 Phosphatase assay for *Pseudomonas fluorescens*

Cultures were kept on nutrient broth-glucose agar slants. P. fluorescens was grown on a synthetic medium (Fraser and Jerrel, 1953) [14] supplemented by 0.0135°/0 Difco yeast extract. The phosphate buffer in Fraser's medium was changed to 0.1 M Tris-HC1, pH 7.4 to provide for the induction of alkaline phosphatase. The yeast extract in this case supplied the minimal concentration of 50 mpmoles orthophosphate per liter which was obligatory for growth.

7.3.2 Preparation of Extracts:

Bacteria were dispersed in 0.1 M Tris-HC1 buffer, pH 7.4, in about one tenth of the original culture volume. After 10 min treatment in the sonic oscillator, cell debris was removed by 20 min centrifugation at 10,000rpm at 20min. The supernatant afforded the crude enzyme solution. In some experiments total phosphatase activity was determined in cells lysed by toluene.

7.3.3 Determination of Enzyme Activity:

Standard reaction mixture (3.0 ml) contained 0.4 M Tris-HC1, pH 8.6, 1.0 mM NPP(p-Nitrophenylphosphate), and enzyme at room temperature. Absorbance of the released p-nitrophenol was read at 420 mp in cuvettes with 1.0 cm light path using the Zeiss Model PMQII spectrophotometer. A unit of enzyme was defined as that amount which hydrolyzed one mµmole NPP per min at standard conditions of assay

7.4 Siderophore detection in *Pseudomonas fluorescens*

A loopful of bacterial culture was inoculated in King's B medium and incubated at 28±2^{0}C for 24 hours. The 24 hours culture was then taken and centrifuged at 8000rpm for 30 minutes. Optical Density for that filtrate was taken at 600 nm under UV spectrophotometer.

7.4.1 Detection of catechol type siderophores in *Pseudomonas fluorescens*

1ml of culture supernatant was mixed with 1ml of 0.5M HCL. To that add 1ml of Nitrate –Molybdate reagent. Yellow colour development indicates the presence of catechol type siderophores. After the addition of few drops NaOH, Pink colour was developed. This pink colour was then turned to red, when NaOH was added in excess.

7.4.2 Separation of siderophores by paper chromatography

Whatmann No.1 filter paper of 10×15cm was taken. 2cm from the bottom of the filter paper place a drop of culture supernatant and place it in the solution which contains 5.7ml glacial acetic acid and 24.3ml Pyridine in 1 litre of water. Then the paper was dried carefully to remove all traces of pyridine and acetic acid. It was then sprayed on both the sides with Chrome Azurol S (CAS) solution. A pink spot appeared on a light blue background indicates the presence of siderophores.

Results

P.fluorescens was isolated from rhizosphere soil and *R.leguminosarum* was isolated from root nodules. Identification of selected isolates based on biochemical characterization as described in Bergey's Manual of Determinative Bacteriology. Some specific tests were carried out for the identification of *P.fluorescens* (Table 1).

The two strains *P.fluorescens* and *R. leguminosarum* were selected for the further evaluation of their growth promoting traits in black gram.

Colony forming units of seed bacterization and soil carrier (CFU/g) were examined for the isolates viability. The results were tabulated in Table 2. Colony forming units for dual inoculation with *R. leguminosarum* and *P.fluorescens* was higher colony forming units than that of individual inoculation of both methods.

The results showed that there was a significant difference in the germination of the plant *Phaseolus mungo* (black gram) inoculated with *P.fluorescens* (B), *R. leguminosarum* (C) and both *P.fluorescens* and *R. leguminosarum* (D) respectively. Among the seed bacterization and soil application, seed bacterization had shown to exhibit early germination than soil application (Table 3). It was observed that the germination of the seeds started on the second day in the case of all treatments. Significant differences were observed among the treatments on the 2nd and 3rd days because the given seed inoculants treatments increased percentage of seed germination than soil application. Co-inoculation of *R.leguminosarum*+ *P.fluorescens* showed highest seed germination percentage after third day (87±0.06% in seed bacterization and 66±0.02% in soil application treatments).

Table 4 shows the growth pattern of the black gram plants raised from the soil application. The height of the plants, shoot length, root length, internodes distance and nodule formation were increased when compared to that of the control plant.

The growth of black gram in different bacterial inoculations was assessed. After 14 days of growth, the maximum height of the shoot length was 3.4±0.02cm in inoculation of *P.fluorescens* and the minimum of 3.0±0.02 cm was observed in control. It was 3.2±0.06 cm in *R.leguminosarum* inoculation and 3.9±0.06 cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens.* Similarly, the maximum height of the root length was observed in 6.0±0.03cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens* and the minimum of 4.2±0.04 cm was observed in control.

Inoculation significantly enhanced the plant height of black gram over uninoculated control. Although co-inoculation caused a significantly increased plant growth as compared to single control. Up to 27.7% increased in plant height was observed in case of co-inoculation as compared to control.

After 40 days of growth, the maximum height of the shoot length was observed in14.7±06 cm in inoculation of *P.fluorescens* and the minimum of 12.7±0.06cm was observed in control. It was 14.7±0.06 cm in *R.leguminosarum* inoculation and 3.9±0.06 cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens.* Similarly, the maximum nodultion was observed in 17±0.06cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens* and the minimum of 8±0.05cm was observed in control (Table 5).

The growth pattern of seed bacterization of black gram plants has been shown in Table 6. The height of the plants means shoot length, root length, internodes distance and nodule formation were highest when compared to that of plants raised from soil application.

In the seed bacterzation method, the black gram growth was observed after 14 days. The maximum height of the shoot length was 4.4±0.06cm in inoculation of *P.fluorescens* and the minimum of 3.0±0.17cm was observed in control. It was 4.2±0.04cm in *R.leguminosarum* inoculation and 5.7±0.06 cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens.* Similarly, the maximum height of the root length was observed in 6.8±0.08cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens* and the minimum of 4.2±0.17cm was observed in control.

In the seed bacterzation method the black gram growth was observed after 40 days. The maximum height of the shoot length was 14.6±0.08cm in inoculation of *P.fluorescens* and the minimum of 12.7±0.00cm was observed in control. It was 13.8±0.08cm in *R.leguminosarum* inoculation and 18.1±0.09 cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens.* Similarly, the maximum height of the root length was observed in 17.8±0.15 cm in the co-inoculation of *R.leguminosarum*+ *P.fluorescens* and the minimum of 14.0±0.15cm was observed in control. Number of root nodule formation was increased enormously in inoculation of *R.leguminosarum* (19±0.06) and co-inoculation of *R.leguminosarum*+ *P.flurescence*(16±0.06).

The results indicated that the maximum plant growth and root nodule formation was obtained in seed bacterization plants than compared to the soil inoculated plants (soil application). Seed bacterization plants showed the best plant growth of black gram than that of soil application. These bio-fertilizers directly coated on the seeds induced the excellent growth of shoot length, root length, increased internodes space and nodule formations due to direct microbial activity of microbes on plant growth significantly when compared to control. So, it is used as a best plant growth promoting co-inoculants than the single inoculants**.**

After 21 days, Dual inoculation was increased root colonization of black gram root than the single inoculants and control. Single inoculation with *P. fluorescens* and *R. leguminosorum* was observed higher colony forming units than control. Table 8 shows increased CFU counts per gram of root segments for the inoculated strains on 21st day. The result showed that root colonization of bacterial inoculants in black gram was higher colony forming units than compared to control.

Isolated strain *Pseudomonas fluorescens* were tested in terms of their applicability to control phytopathogenic strains of *Fusarium solani.* Higher zones of inhibition were observed on 80µl of culture that may be the direct influence of metabolites produced by *P. fluorescens* that inhibited the mycelial growth of tested fungi (Plate 1).

Production of HCN of *P.fluorescens* was confirmed by yellow to deep brown colour on the picric acid dipped filter paper ends. Fig 2 shows phosphatase activity of *P. fluorescens*. Maximum phosphatase activity of *P. fluorescens* was found that 28U/ml after 3days of incubation.

A pink colour spot appeared on a light blue background of filter paper, which indicated the production of siderophore by *P. fluorescens*.

Discussion

In the present study, *Pseudomonas fluorescens* was isolated from rhizosphere soil and *Rhizobium leguminosarum* was isolated from root nodule of legume plants. *Pseudomonas fluorescens* showed excellent fluorescence under UV light on King's B medium. Similarly, (Kremer *et al*., 1990) [15] isolated *Pseudomonas* strains showed fluorescence under UV light on King's B medium. These characteristics were regarded as taxonomically useful characteristics for *Pseudomonas* (Palleroni *et al.*, 1973) [16].

In the present study, seed bacterization increased bacterial colonization around a seed and plant root than soil application. Bacterial colonization of *Pseudomonas fluorescens, Rhizobium leguminosarum* and dual inoculation

with *P.fluorescens and R. leguminosarum* around a seed were found to be 2.6×10^6CFU/seed, 1.9×10^7CFU/seed and 3.5×10^7CFU/seed respectively. The seed bacterization promoted early germination than soil application and uninoculated seed. Among the three treatments, dual inoculation has shown increased colonization. Burdman *et al.* (1998) reported that co-inoculation of several legumes with *Rhizobium sp* induced plant growth. (Burdman *et al.*, 1998) [17] reported that increased in root and shoot length in rice plant as compared to control might be due to *P. fluorescens* because of their plant growth promoting activity.

These PGPR enhance the growth of plants by increasing nodulation, nitrogen fixation and phosphate solubilization (Kaushal *et al.*, 2013; Vivas *et al.*, 2005) [18, 19] reported that PGPR isolated from cadmium contaminated soil, increased the nodulation of clover plants growing in this soil. *Pseudomonas sp.* exhibits an effective biocontrol including direct antagonism and induction of plant resistance. Similarly, in the present work also *R.leguminosarum* increased nodulation in dual inoculation of black gram than single inoculation.

Bacterial inoculation has a significant effect on the growth of black gram. After 21 days, dual inoculation was increased root colonization of black gram root than the single inoculants and control. Similarly, (Kennedy, 1998) [20] has demonstrated that great array of root-microbe interactions occurs in rhizosphere soil. Inoculation of *Rhizobium, Pseudomonas putida, P.fluorescens* and *Bacillus cereus* increased significantly the plant growth and nodulation of faba bean (Tilak *et al*., 2006) [21]. Mixed inoculation of *Vicia faba L.* with four different *Rhizobium / Azospirillum* and *Rizobium / Azotobacter* combinations led to changes in total content, concentration and distribution of the mineral macro-and micronutrients, when with respect to plants inoculated with *Rhizobium* lonely (Rodelas, 1999) [22].

Nabrdalik and Grata, (2014) [23] reported that the influence of metabolites from *Pseudomonas fluorescens* on the growth of four pathogenic strains of *Rhizoctonia solani* infesting sugar beet. The tests showed that growth inhibition of the mycelium depends not only on the type of metabolites produced by a specific bacterial strain but also on the length of culturing. In the present work, the antifungal activity of *P. fluorescens* showed increased amount of metabolite from different growth periods of *Pseudomonas fluorescens* that greatly inhibited the mycelium of *F. solani.* Plant growth promoting bacteria are those present in the rhizosphere and improve the growth of the plant directly or indirectly (Afzal and Bano, 2008) [24]. A considerable number of bacterial species from the rhizosphere have been isolated and their efficiency for improving plant growth has been assessed (Amor *et al.*, 2008) [25].

As agricultural production strengthened over the past few decades, farmers became more increasing use of chemical inputs causes several negative effects, i.e., development of pathogen resistance to the applied agents and their non target environmental impacts (Biswas *et al*., 2000) [26]. This leads to the need of frequent application of these chemicals which are costly and also environmentally undesirable [27]. *Pseudomonas fluorescens* is one of the fluorescent Pseudomonads that secrete pyoverdin. Pyoverdin is a yellowish-green fluorescent siderophore involved in high affinity transport of iron into the cell (Arshad and Frankenberger, 1993) [28]. Kinzel *et al.* (1998) [29] have investigated the specific stimuli for siderophores production in local strains of *Pseudomonas fluorescens* under different Fe levels.

Conclusion

The experimental findings indicated that rhizosphere strains of *P. fluorescens* is positively interact with the *R. leguminosarum* (legume symbiosis), promoting black gram plant growth and induced nodulation. The co-inoculation of these inoculants not only enhanced the plant growth but also produced siderophores, antibiosis against fungi, hydrogen cyanide etc. From the agricultural view points, co-inoculants should increase plant productivity and also maintain environmental quality.

Table 1: Results of biochemical characteristics of *P. fluorescens*

S.No.	Characteristic	*Pseudomonas fluorescens*
1	Growth at 4^0C	+
2	Growth at 40^0C	-
3	Fluorescin	+
4	Pyocyanin	-
5	Starch hydrolysis	+
6	Lipolysis	-
7	Gelatin hydrolysis	+

Table 2: Colony forming units of bacterized seeds of black gram and soil

S. No.	Source of bacteria	CFU/ g Bacterized Seed	CFU/ g Treated soil
1	*P. fluorescens*	2.6×10^7	5.7×10^7
2	*R. leguminosarum*	1.9×10^7	4.7×10^7
3	*P. fluorescens + R. leguminosarum*	3.5×10^7	14.7×10^7

Table 3: The effect of the seed bacterization and soil application treatments on the number of germinated seeds.

Treatments		Days (germination percentage)		
		2	3	4
Seed bacterization	*R. leguminosarum*	20±0.06	50±0.04	92±0.04
	P.flurescence	41±0.02	63±0.03	83±0.04
	R.leguminosarum+ P.flurescence	50±0.06	87±0.06	100±00
Soil application	*R. leguminosarum*	16±0.04	45±0.06	83±0.06
	P.flurescence	33±0.04	54±0.04	79±0.02
	R.leguminosarum+ P.flurescence	41±0.06	66±0.02	92±0.02

Table 4: Effect of soil application of bacterial inoculants on growth patterns of black gram (after 14 days)

S No	Organisms	Plant height (cm)	Shoot length (cm)	Root length (cm)	Inter node (cm)	Nodule formation
1.	Control	7.2±0.06	3.0±0.02	4.2±0.04	-	-
2.	*P.fluorescens*	8.9±0.03	3.4±0.02	5.5±0.04	-	-
3.	*R.leguminosarum*	8.6±0.06	3.2±0.06	5.4±0.05	-	-
4.	*P.fluorescens+ R.leguminosarum*	9.2±0.02	3.9±0.06	6.0±0.03	-	-

Table 5: Effect of soil application of bacterial inoculants on growth patterns of black gram (after 40 days)

S.No	Organisms	Plant height (cm)	Shoot length (cm)	Root length (cm)	Inter node (cm)	Nodule formation
1.	Control	26.7±0.02	12.7±0.06	14.0±0.04	0.9±0.04	8±0.05
2.	*P.fluorescens*	30.5±0.02	14.7±0.06	16.8±0.04	1.7±0.04	9±0.06
3.	*R.leguminosarum*	28.9±0.05	13.9±0.05	15.0±0.02	1.3±0.04	14±0.06
4.	*P.fluorescens*+ *R.leguminosarum*	35.6±0.06	18.8±0.02	18.8±0.02	1.8±0.03	17±0.06

Table 6: Effect of seed bacterization of bacterial inoculants on growth patterns of black gram (after 14 days)

S. No	Organisms	Plant height (cm)	Shoot length (cm)	Root length (cm)	Inter node (cm)	Nodule formation
1.	Control	7.2±0.15	3.0±0.17	4.2±0.17	-	-
2.	*P.fluorescens*	9.9±0.08	4.4±0.06	5.5±0.17	-	-
3.	*R.leguminosarum*	9.7±0.06	4.2±0.04	5.5±0.08	-	-
4.	*P.fluorescens*+ *R.leguminosarum*	11.8±0.06	5.7±0.06	6.8±0.08	-	-

Table 7: Effect of seed bacterization of bacterial inoculants on growth patterns of black gram (after 40days)

S. No	Organisms	Plant height (cm)	Shoot length (cm)	Root length (cm)	Inter node (cm)	Nodule formation
1.	Control	26.7±0.06	12.7±0.00	14.0±0.15	0.9±0.01	8±0.06
2.	*P.fluorescens*	31.4±0.07	14.6±0.08	16.8±0.15	1.7±0.01	10±0.06
3.	*R.leguminosarum*	30.3±0.00	13.8±0.08	16.5±0.15	1.3±0.02	19±0.06
4.	*P.fluorescens*+ *R.leguminosarum*	37.9±0.06	18.1±0.09	17.8±0.15	1.9±0.02	16±0.06

Table 8: Root colonization of bacterial inoculants in black gram (after 21 days)

S.No	Organisms	CFU/g
1.	Control	4.7×10^2
2.	*P.fluorescens*	1.7×10^4
3.	*Rhizobium sp*	1.2×10^4
4.	*P.fluorescens + Rhizobium sp*	12.5×10^4

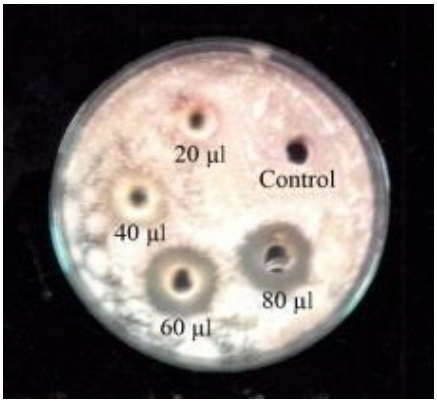

Plate 1: *In vitro* tests for antagonism of *P.fluorescens* against *Fusarium solani*

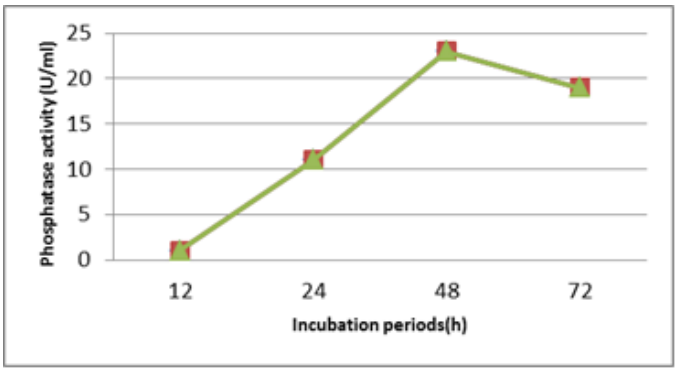

Fig 2: Phosphatase activity of *P. fluorescens*

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