

## Interaction of *Pseudomonas fluorescens* with *Rhizobium* for Their Effect on the Management of Peanut Root Rot

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A biocontrol agent (*Pseudomonas fluorescens*) and a phytostimulator (*Rhizobium*) have been shown to have beneficial effects on plant growth and health. The study of plants inoculated with *Pseudomonas* and *Rhizobium* requires special attention because of the possibility that these agents may influence each other. Our study was conducted to test the effect of these inoculants on co-inoculation in peanut to control root rot, a severe soilborne disease caused by *Macrophomina phaseolina*. One fluorescent pseudomonad strain, Pf 1, which effectively inhibited the mycelial growth of *M. phaseolina* under *in vitro* conditions, was studied for its compatibility with the biofertilizer bacterial strain *Rhizobium* TNAU 14. Dual culture and colorimetric studies indicated the existence of a positive interaction between the microbial inoculants. However, glasshouse and field studies showed seed treatment and soil application of *Pseudomonas fluorescens* Pf 1 to be the most effective treatment in reducing root rot incidence and improving the crop vigor index, in comparison with treatments in which both inoculants were applied.

KEY WORDS: *Pseudomonas fluorescens*; *Rhizobium*; interaction; peanut; *Macrophomina phaseolina*; root rot.

### INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a major oilseed and food crop of the semiarid tropics. Dry root rot caused by the soilborne pathogen *Macrophomina phaseolina* (Tassi) Goid causes extensive yield loss to the crop. The existing commercial cultivars of peanut do not provide sufficiently high levels of disease resistance. Although fungicides can control the disease, their use raises the cost of production and increases environmental pollution and damage to the ecosystem. The pressure to reduce the use of these chemicals has led to increased interest in other approaches, including microbiological control, *i.e.*, the use of beneficial microorganisms for protection against harmful organisms. Heterotrophic rhizobacteria of the *Pseudomonas fluorescens* type have been used successfully for biological control of several plant pathogens (2,7,19). The inoculation of symbiotic N-fixing *Rhizobia* in seeds enhances the nodulation capacity of plant roots of legumes and thereby promotes the plant yields and N availability in soil (14). *Rhizobia* are aerobic, heterotrophic, non-spore-forming, soil inhabitants and able to form nodules on roots of host plants symbiotically and to fix atmospheric nitrogen. However, this response is not always uniform because of the microflora associated with root nodules and the

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presence of microbial antagonists in the soil. Therefore, studies of the interaction between the biological agent *P. fluorescens* and the phytostimulator *Rhizobium* are important particularly with reference to legumes.

In the present study we screened several strains of fluorescent pseudomonads for their efficacy in inhibiting *M. phaseolina*, the causal agent of peanut dry root rot under *in vitro* conditions. In addition, the interaction of the effective strain with *Rhizobium* TNAU 14, the biofertilizer bacterial strain commonly used as a seed treatment for peanut in Tamil Nadu, India, was investigated in dual culture and colorimetric studies. Also, a talc-based formulation of the effective strain was developed and its efficacy was studied in association with *Rhizobium* for the control of dry root rot of peanut under glasshouse and field conditions.

## MATERIALS AND METHODS

**Isolation of pathogen and fluorescent pseudomonads** An isolate of *M. phaseolina* was obtained from infected peanut stems and roots using potato dextrose agar (PDA) medium. The pathogenicity of the fungus was tested by applying the fungus grown in sand – maize medium (13). Five peanut (Co 2) seeds were sown in a 30-cm-diam earthen pot containing the fungal culture incorporated in sterilized soil at a ratio of 1:19 (sand-maize inoculum: soil). The root rot incidence was assessed and re-isolation of the pathogen from the rotted plant was done using PDA.

Rhizoplane-colonizing fluorescent pseudomonads were isolated from fresh roots of peanut, carrot, banana, tapioca, pepper, rice, and forest trees grown in several geographic areas of Tamil Nadu, India (Table 1). After vigorous shaking of excised roots to remove all but tightly adhering soil, root segments (1 g) were shaken in 100 ml of sterile distilled water for 15 min and serial dilutions were made up to  $10^6$ . Fluorescent pseudomonads were isolated using King's medium B (KMB; 9) and fluorescent colonies were detected by viewing under UV light.

***In vitro* screening of *P. fluorescens* strains against *M. phaseolina*** The efficacy of the fluorescent pseudomonad strains was tested by streaking the bacteria at one side of the petri dish containing PDA. A 4-mm mycelial disc from 5-day-old *M. phaseolina* culture in PDA was placed on the opposite side in the petri dish perpendicular to the bacterial streak. The growth of the fungus was inhibited when it grew toward the bacterial colony. The inhibition zone was measured from the edge of mycelium to the bacterial colony edge after 7 days of incubation. The experiments were carried out in a completely randomized design and three replications were maintained for each treatment. The bacterial strains, which showed inhibition against *M. phaseolina*, were identified based on the following biochemical tests, *viz.*, production of fluorescent pigment (9), gelatin liquefaction, nitrate reduction, arginine dihydrolase, levan formation and growth at 4° and 41°C, and different carbon source utilization (6). One fluorescent pseudomonad strain, Pf 1, which effectively inhibited the growth of *M. phaseolina*, was used to develop a talc-based powder formulation, following the methods described by Vidhyasekaran and Muthamilan (16). Briefly, a loopful of bacterial strain was inoculated into KMB broth and grown in a rotary shaker at 150 rpm for 48 h at room temperature ( $28\pm 2^\circ\text{C}$ ). One kg of talc powder (montmorillonite) was taken in a metal tray under aseptic conditions and its pH was adjusted to neutral by adding  $\text{CaCO}_3$  at the rate of  $15\text{ g kg}^{-1}$ . Then 10 g of carboxyl methyl cellulose was added to 1 kg of talc, mixed well and the mixture was autoclaved for 30 min at  $120^\circ\text{C}$  on each of

two consecutive days. Then 400 ml of the bacterial suspension containing  $9 \times 10^8$  colony forming units (cfu)  $\text{ml}^{-1}$  was added to a carrier-cellulose mixture under aseptic conditions. After drying (35% moisture content) overnight under aseptic conditions, the mixture was packed in a polypropylene bag, sealed and stored at room temperature ( $25 \pm 2^\circ\text{C}$ ). At the time of application, the population of bacteria in the formulation was  $9 \times 10^8$  cfu  $\text{g}^{-1}$  of talc powder.

***P. fluorescens*–*Rhizobium* interaction by the dual culture method** The efficient strain (Pf 1), which effectively inhibited the growth of *M. phaseolina* and *Rhizobium* (TNAU 14), is recommended as seed treatment for peanut and is available at the Dept. of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India, was used throughout these studies. Interaction between the inoculates was studied under *in vitro* conditions as follows: Bacterial suspension was made by suspending the cells (Pf 1) in KMB broth. The density was measured spectrophotometrically at 595 nm and adjusted to  $10^7$  cfu  $\text{ml}^{-1}$ . The strain was spot-inoculated on KMB agar plates by pipetting two droplets of 5  $\mu\text{l}$  of the bacterial suspension. The spot-inoculated plates were incubated at  $27^\circ\text{C}$  for 48 h. Subsequently, a suspension of the target *Rhizobium* strain ( $10^7$  cfu  $\text{ml}^{-1}$ ) was atomized over the spot-inoculated plates. After an additional incubation period of 24 h at  $27^\circ\text{C}$ , zones of growth inhibition of the target strain around the spot-inoculated strains were measured. Inhibition is expressed as the diameter of the inhibition zone divided by the diameter of the spot-inoculated colony.

***P. fluorescens*–*Rhizobium* interaction by the spectrophotometric method** Fifty ml of nutrient broth was sterilized and inoculated with 0.5 ml of the cell suspensions of Pf 1 and TNAU 14 each at  $3 \times 10^7$  cfu  $\text{ml}^{-1}$ . The flasks were shaken continuously in an orbital shaker for 72 h. The populations of Pf 1 and TNAU 14 were enumerated separately through serial dilution and plating in nutrient agar medium. The fluorescent colonies of Pf 1 were detected by viewing under UV light. The intensity of growth was assessed by observing the optical density of the broth in a colorimeter at 595 nm. Cultures of Pf 1 and TNAU 14 grown separately in nutrient broth served as control.

To study the influence of culture filtrate of *P. fluorescens* on the growth of *Rhizobium*, Pf 1 was inoculated to flasks containing sterilized nutrient broth and incubated for 7 days. The contents were filtered and passed through a membrane filter (0.45  $\mu\text{m}$ ). The filtrate was incorporated into nutrient broth at 20% (v/v) and inoculated with 0.5 ml of *Rhizobium* cell suspension ( $3 \times 10^7$  cfu  $\text{ml}^{-1}$ ). After an incubation period of 72 h in a rotary shaker, the population of TNAU 14 was estimated as described above. The influence of TNAU 14 culture filtrate on the growth of Pf 1 was assessed. The experiments were repeated once with consistent results and the data presented are the means of three replications.

**Interaction of *P. fluorescens* with *Rhizobium* sp. upon challenge inoculation with the pathogen in a glasshouse** Glasshouse trials were conducted at the Dept. of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, during August–October 1999. Peanut seeds of the susceptible cultivar, Co 2, were surface sterilized with 2% sodium hypochlorite solution for 2 min and treated with the talc-based formulation of Pf 1 at 4 g  $\text{kg}^{-1}$  seed and dried in the shade for 2 h. One hundred mg of the formulated product of Pf 1 (2.5 kg talc-based formulation mixed with 50 kg of farmyard manure) was given as soil application per pot at the time of sowing. A peat-based formulation of TNAU 14 was used for seed treatment at 6 g  $\text{kg}^{-1}$  seed. Control plants without any treatment were also

maintained. Five seeds were sown in individual 30-cm-diam earthen pots containing the fungal culture incorporated into 5 kg steam-sterilized soil at a ratio of 1:19 (sand-maize, inoculum : soil), with 250 g per pot (5%). All treatments were replicated three times in a randomized block design.

The plants were removed at 45 and 90 days after sowing (DAS) from each pot and bacterial populations in the rhizosphere were assessed by the method described by Papavizas and Davey (12) for *P. fluorescens* and by Subba Rao (14) for *Rhizobium*. The fluorescent colonies of *P. fluorescens* were viewed under UV light and the populations were expressed for 100 g of soil. Observations on vigor index and root rot incidence (%) were recorded at 45 and 90 DAS, respectively. The pinkish nodules and kernel yield ( $\text{kg ha}^{-1}$ ) were recorded at the time of harvest for all treatments. The pinkish nodules were counted after washing the root zone under running tap water and expressed in numbers per plant.

**Field evaluation of interaction on growth, yield measurements and dry root rot incidence** Two field experiments (garden land) were conducted under irrigated conditions with three replications in a randomized block design during 1999 and 2000 at the Agricultural Research Station, Aliyar Nagar, Tamil Nadu, which is an endemic location for dry root rot. A standard plot size of  $5 \times 4 \text{ m}^2$  was maintained for all the treatments. Peanut seeds of susceptible cv. Co 2 were used for the studies. Seed treatment (ST) and soil application (SA) of Pf 1 and seed treatment of TNAU 14 were carried out as described for the glasshouse studies. An untreated control was also maintained. Agronomic and cultural practices were as recommended to farmers. The recommended dose of fertilizer (17:34:54 kg of NPK  $\text{ha}^{-1}$ ) was applied to the field. Crop vigor index and natural root rot incidence were assessed on 20 plants selected at random from each plot at 45 and 90 DAS, respectively. The pinkish nodules and kernel yield ( $\text{kg ha}^{-1}$ ) were recorded at the time of harvest for all treatments.

**Statistical analysis** The data on percentages were arcsine transformed and analysis of variance was performed with transformed values (4). The treatment means were compared by Duncan's multiple range test (DMRT). The package used for analysis was IRRISTAT version 92-1 developed by the Biometrics Unit of the International Rice Research Institute, The Philippines.

## RESULTS

**Isolation and screening of *P. fluorescens* strains against *M. phaseolina*** Ten different fluorescent pseudomonad strains were isolated from fresh roots of peanut, carrot, banana, tapioca, pepper, rice and forest trees grown in several geographic areas of Tamil Nadu, India. In the preliminary screening of *Pseudomonas* strains for their antagonistic activity against *M. phaseolina*, under *in vitro* conditions, strain Pf 1 was identified as the most effective bacterial antagonist, with an inhibition zone of 11.6 mm (Table 1).

***In vitro* interaction of *P. fluorescens* with *Rhizobium*** Based on the performance from the above studies, strain Pf 1 was selected and tested for its interaction with TNAU 14. Dual culture studies on the interaction of *Pseudomonas* strain Pf 1 with TNAU 14 under *in vitro* conditions revealed no mutual inhibition of growth. Furthermore, colorimetric studies showed that the individual growth of Pf 1 and TNAU 14 was not affected significantly compared with control when they were dual-cultured in nutrient broth. The O.D. values of Pf 1 (0.46) and *Rhizobium* (0.39) observed in the present studies were at par with their respective control treatments when cultured. Studies of the influence of Pf 1 culture filtrate

TABLE 1. Efficacy of different *Pseudomonas fluorescens* strains in inhibiting growth of *Macrophomina phaseolina* under *in vitro* conditions

<i>P. fluorescens</i> strain	Crop rhizosphere	Inhibition zone (mm)
Pf 1	Black gram	11.6 a <sup>z</sup>
PFKO 1	Paddy	4.7 cd
PFBS 1	Paddy	3.2 bc
PFATR 1	Tapioca	5.3 de
PFMDU 1	Paddy	6.5 e
FP 7	Paddy	9.0 f
PFNA 1	Banana	5.6 de
PFNL 1	Forest trees	4.0 bcd
PFKO 2	Pepper	6.8 e
PFRA 1	Carrot	2.5 b
Control	-	0.0 g

<sup>z</sup> Means followed by a common letter do not differ significantly by Duncan's multiple range test ( $P = 0.05$ ).

TABLE 2. Interaction of *Pseudomonas fluorescens* (Pf 1) and *Rhizobium* on vigor index of peanut and survivability of the inoculants in the rhizosphere under glasshouse conditions, 45 and 90 days after sowing (DAS)

Treatments <sup>z</sup>	Vigor index <sup>y</sup>	Population per 100 g of soil ( $\times 10^6$ )			
		45 DAS		90 DAS	
		<i>Pseudomonas</i> <sup>w</sup>	<i>Rhizobium</i> <sup>w</sup>	<i>Pseudomonas</i>	<i>Rhizobium</i>
ST - Pf 1	2503.6 cd <sup>z</sup>	21.6 (1.3) ab	0.0 (2.0) d	40.5 (1.6) a	0.0 (2.0) c
ST - <i>Rhizobium</i>	2371.8 bc	0.0 (2.0) f	25.4 (1.4)c	0.0 (2.0) e	30.1 (1.4) b
SA - Pf 1	2173.6 b	24.3 (1.3) c	0.0 (2.0) d	47.8 (1.6)bc	0.0 (2.0) c
ST + SA - Pf 1	3248.6 g	30.0 (1.4) e	0.0 (2.0) d	56.1 (1.7) d	0.0 (2.0) c
ST - <i>Rhizobium</i> + ST - Pf 1	2861.4 ef	20.8 (1.3) a	22.2 (1.3) b	39.2 (1.5) a	26.9 (1.4) a
ST - <i>Rhizobium</i> + SA - Pf 1	2693.8 de	23.1 (1.3) bc	23.1 (1.3) b	45.5 (1.6) b	28.0 (1.4) ab
ST + SA - Pf 1 + ST - <i>Rhizobium</i>	3049.2 fg	27.4 (1.4) d	20.0 (1.3) a	51.4 (1.7) cd	25.0 (1.4) a
Control	1813.5 a	0.0 (2.0) f	0.0 (2.0) d	0.0 (2.0) e	0.0 (2.0) c

<sup>z</sup> ST - seed treatment (Pf 1, 10 g kg<sup>-1</sup> seed; *Rhizobium*, 6 g kg<sup>-1</sup> seed); SA - soil application (Pf 1, 100 g per pot).

<sup>y</sup> Vigor index: Seed germination (%)  $\times$  [root length + shoot length].

<sup>z</sup> Within columns, means followed by a common letter do not differ significantly by Duncan's multiple range test ( $P=0.05$ ).

<sup>w</sup> Logarithmic transformation of the data was carried out prior to analysis by DMRT for *Pseudomonas* and *Rhizobium* populations.

when incorporated into nutrient broth, over the growth and population of TNAU 14 and *vice versa*, found the filtrates to be significantly non-inhibitory compared with control values.

**Root rot development and growth measurements under glasshouse conditions** In the pot culture experiment, application of Pf 1 as ST and SA increased the vigor index of the crop to the maximum (3248.6) (Table 2). Although moderate nodulation (13.6) was observed, the treatment exhibited greater reduction in root rot incidence (29.1%) and maximum kernel yield (15.73 g per plant) (Table 4). The maximum nodulation (28.3) influenced by seed treatment with TNAU 14 alone had less impact on root rot incidence (52.2%) and kernel yield (9.45 g per plant) (Table 3). Studies on the survival of Pf 1 and TNAU 14 in the rhizosphere of peanut (Table 2) indicated maximum population of Pf 1 when the same was applied as ST and SA ( $30.0 \times 10^6$  cfu) and of *Rhizobium* when applied as ST ( $25.4 \times 10^6$  cfu). The population increased to 56.1 and  $30.1 \times 10^6$  cfu at the end of the study period (90 DAS) (Table 2). Although applications of Pf 1 in different combinations with TNAU 14 differ significantly in their effect over control, they were observed to be next in effectiveness only to ST and SA of Pf 1. Among the combinations, ST and SA of Pf 1 with ST of TNAU 14 showed the maximum vigor index (3049.2) (Table 2), maximum nodulation per plant (25.6), highest kernel yield (13.67 g per plant), and minimum root rot incidence (33.3%) (Table 3).

TABLE 3. Interaction of *Pseudomonas fluorescens* (Pf 1) and *Rhizobium* on native nodulation, root rot incidence and kernel yield

Treatments <sup>z</sup>	Glasshouse studies			Field studies					
	Root rot (%) <sup>y</sup>	Nodules/plant	Yield (g plant <sup>-1</sup> )	Trial I			Trial II		
				Root rot (%) <sup>y</sup>	Nodules/plant	Yield (kg ha <sup>-1</sup> )	Root rot (%) <sup>y</sup>	Nodules/plant	Yield (kg ha <sup>-1</sup> )
ST - Pf 1	45.8 (42.6) e	10.6 b	10.27 cd	44.5 d	13.5 c	866 cde	40.7 d	11.1 a	850 d
ST - <i>Rhizobium</i>	52.2 (46.3) f	28.3 f	9.45 bc	53.3 f	41.6 a	812 de	55.1 f	38.3 f	799 cd
SA - Pf 1	53.0 (46.7) f	8.0 a	8.81 ab	49.6 e	13.4 c	780 de	45.3 e	10.5 a	760 bc
ST + SA - Pf 1	29.1 (32.6) a	13.6 c	15.73 g	25.5 a	14.4 c	1304 a	23.1 a	12.5 b	235 a
ST - <i>Rhizobium</i> + ST - Pf 1	38.6 (38.4) c	22.9 d	12.03 e	34.1 b	26.8 b	964 bc	35.4 bc	25.2 d	990 f
ST - <i>Rhizobium</i> + SA - Pf 1	41.6 (40.2) d	22.6 d	11.08 de	38.2 c	28.6 b	920 cd	36.3 c	30.4 e	906 e
ST + SA - Pf 1 + ST - <i>Rhizobium</i>	33.3 (35.2) b	25.6 e	13.67 f	32.5 b	26.2 b	1085 b	33.5 b	22.8 c	1012 f
Control	46.5 (48.7) g	6.8 a	8.04 a	64.5 g	10.6 c	740 e	58.2 g	9.9 a	720 b

<sup>z</sup> ST - seed treatment (Pf 1, 10 g kg<sup>-1</sup> seed; *Rhizobium*, 6 g kg<sup>-1</sup> seed); SA - soil application (Pf 1, 2.5 kg ha<sup>-1</sup>).

<sup>y</sup> Arcsine transformation of the data was carried out prior to analysis by Duncan's multiple range test for root rot incidence.

<sup>z</sup> Within columns, means followed by a common letter do not differ significantly by DMRT ( $P = 0.05$ ).

TABLE 4. Interaction of *Pseudomonas fluorescens* (Pf 1) and *Rhizobium* on plant biometrics of peanut under field conditions

Treatments <sup>z</sup>	Trial I		Population per 100 g of soil ( $\times 10^6$ )				Trial II		Population per 100 g of soil ( $\times 10^6$ )			
	Vigor index <sup>y</sup>		45 DAS		90 DAS		Vigor index		45 DAS		90 DAS	
			Pf 1 <sup>z</sup>	<i>Rhizobium</i> <sup>z</sup>	Pf 1	<i>Rhizobium</i>			Pf 1	<i>Rhizobium</i>	Pf 1	<i>Rhizobium</i>
			ST - Pf 1	2274.3 b <sup>w</sup>	40.8 e	10.3 d			42.4 e	18.3 d	2109.8 bc	42.3 e
ST - <i>Rhizobium</i>	2236.8 b	13.8 f	52.5 a	14.8f	57.5 a	1995.4 b	10.5 h	44.8 a	16.5 f	53.8 a		
SA - Pf 1	2180.6 ab	49.2 c	10.5 d	50.1 d	18.4 d	1820.0 a	48.2 c	16.5 d	53.3 c	15.8 e		
ST + SA - Pf 1	3103.7 d	59.7 a	11.3 d	62.1 a	17.8 d	3354.6 e	55.3 a	15.6 de	58.3 a	12.8 g		
ST - <i>Rhizobium</i> + ST - Pf 1	2514.8 bc	39.3 e	37.0 b	41.9 e	43.0 c	2600.9 d	40.4 f	34.2 c	42.9 e	37.8 c		
ST - <i>Rhizobium</i> + SA - Pf 1	2306.5 b	47.3d	38.6 b	53.2 c	44.9 b	2197.4 c	45.1 d	36.6 b	46.9 d	40.3 b		
ST + SA - Pf 1 + ST - <i>Rhizobium</i>	2850.2 cd	51.5 b	37.0 b	56.8 b	42.8 c	2712.2 d	49.5 b	35.8 b	54.6 b	36.6 d		
Control	1732.5 a	13.5 f	13.9 c	13.8 f	17.5 d	1804.9 a	12.1 g	14.5 e	17.6 f	15.0 ef		

<sup>z</sup> ST - seed treatment (Pf 1, 10 g kg<sup>-1</sup> seed; *Rhizobium*, 6 g kg<sup>-1</sup> seed); SA - soil application (Pf 1, 2.5 kg ha<sup>-1</sup>).

<sup>y</sup> Vigor index: Seed germination (%)  $\times$  [root length + shoot length].

<sup>z</sup> Logarithmic transformation of the data was carried out prior to analysis by Duncan's multiple range test for *Pseudomonas* and *Rhizobium* populations. Interaction: treatment  $\times$  bacteria  $\times$  days (LSD = 5%), Trial I = 1.70; Trial II = 1.25.

<sup>w</sup> Within columns, means followed by a common letter do not differ significantly by DMRT ( $P = 0.05$ ).

**Microbial interaction under field conditions** Results from both of the field trials indicated that the talc-based formulation of *Pseudomonas* strain Pf 1 performed similarly in both trials (1999 and 2000). The plots that received ST and SA of Pf 1 had the highest rhizosphere population, the lowest root rot incidence and the maximum kernel yield (Table 3). Enhanced plant growth was also promoted under field conditions (Table 4). The performance of the combined application of Pf 1 and TNAU 14 over microbial survival in the crop rhizosphere, root rot incidence and kernel yield was similar to that observed in the glasshouse studies.

## DISCUSSION

The ecological impact of microbial inoculants in soil has often been characterized in terms of size and composition of specific microbial groups. However, this approach does not provide a comprehensive view of the impact of an inoculant on the function of a soil ecosystem in playing a significant role in disease management. *Pseudomonas fluorescens* is a common rhizobacterium used as a biocontrol agent with definite plant growth-promoting activity, it can be easily isolated from the rhizosphere of various crops, and has been widely used for the management of plant diseases (11,17,18). In the present study its interaction was studied with *Rhizobium*, a biofertilizer bacterium commonly used as a seed treatment for legumes. Earlier, Jagjeet Singh and Lodha (8) studied the antagonistic activity towards *Rhizobium japonicum* of microflora isolated from soybean root nodules. *In vitro* studies of the effect of *P. fluorescens* on *Rhizobium* growth showed the existence of a positive interaction between them, with no significant reduction in growth of either bacterial inoculant. However, the combined application of the efficient strain of *P. fluorescens* Pf 1 with *Rhizobium* under glasshouse and field conditions had some negative impact on the inoculants' survival in a crop rhizosphere, although the combined application was better than the control treatments. This was evident from the fact that application of Pf 1 and *Rhizobium* as individual inoculants showed maximum crop vigor index and kernel yield with minimum disease incidence, as compared with their combined application. Since it is well known that in the soil environment organisms sharing the same ecological niche ought to influence and modify the effects of each other, co-inoculation of *P. fluorescens* with *Rhizobium* might have modified their effects mutually, and thus their efficiencies might have been reduced moderately, contrary to *in vitro* studies. However, some workers have reported compatibility and even an occasional synergism of *Rhizobium* with biocontrol agents. Harman *et al.* (5) reported a similar number of root nodules in plants grown from seeds treated with *Rhizobium* alone or *Rhizobium* + *Trichoderma*. It is of interest to note that even upon challenge inoculation with a pathogen, plants treated with *P. fluorescens* or *Rhizobium* either alone or in combination had minimum disease incidence compared with untreated control plants. Furthermore, the plants grew faster and greener, with longer roots and shoots than the untreated plants. Thus, treatment with *P. fluorescens* and/or *Rhizobium* played a dual role – by reducing disease incidence and promoting plant growth, resulting in increased biomass and yield. It has been established that fluorescent pseudomonads enhance plant growth in several ways, *viz.*, producing plant growth regulators, such as gibberellins, cytokinins and indole acetic acid, which can directly or indirectly modulate the plant growth and development (1,3,10). Furthermore, one can understand that the increased productivity of the plant results in a large part from the suppression of deleterious microorganisms and soilborne pathogens by *P. fluorescens*.

In conclusion, our findings indicate that as a result of microbial colonization by two different bacteria, modifications of structure and ecology of the microbial community occurred. Therefore, their potentials could not be exploited fully under glasshouse and field conditions in the management of peanut root rot, although that was observed under *in vitro* conditions. An understanding of these effects as part of ecosystem processes is essential for obtaining maximum plant growth and health within the context of soil–plant system sustainability.

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