International Journal of Plant Breeding and Genetics ISSN 5756-2148 Vol. 6 (7), pp. 001-012, July, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Biocontrol of wilt disease complex of pigeon pea (*Cajanus cajan* (L.) Millsp.) by isolates of *Pseudomonas* spp.

Z. A. Siddiqui* and U. Shakeel

Department of Botany, Aligarh Muslim University, Aligarh-202002, India.

Accepted 10 April, 2019

Biocontrol of wilt disease complex of pigeon pea, caused by *Meloidogyne incognita*, *Heterodera cajani* and *Fusarium udum*, was studied using 21 isolates of fluorescent Pseudomonads isolated from pathogen suppressive soils. The isolates Pf 718, Pf 719 and Pf 736 of *Pseudomonas fluorescens* and Pa 737 of *P. aeruginosa* caused 79, 84, 87 and 93% reductions in hatching of *M. incognita*, and showed inhibition in the growth of *F. udum* in the dual inoculation. Isolate Pf736 caused 309, 9 and 78% increases in seedling growth, phosphate solubilization and IAA production respectively and also showed moderate HCN production. Isolate Pa737 was the best to colonize roots of pigeon pea followed by Pf736. The effects of these four isolates (Pf718, Pf719, Pf736 and Pa737) were studied on the wilt disease complex both in mono and multi-pathogenic combinations. The isolates Pf736 caused greater increase in plant growth and higher reduction in nematode multiplication and wilting index followed by Pa737, Pf718 and Pf719. The use of these isolates along with *Rhizobium* (pigeon pea strain) further increased plant growth and reduced nematode multiplication and wilting index. Twelve isolates production of siderophores in Chrome Azurol S (CAS) agar medium. The results suggest that *P. fluorescens* Pf736 along with *Rhizobium* may be used for the management of wilt disease complex of pigeon pea.

Keywords: Fusarium udum, Heterodera cajani, Meloidogyne incognita, Pseudomonas aeruginosa, P. fluorescens, Rhizobium.

INTRODUCTION

Heterodera cajani Koshy, *Meloidogyne incognita* (Kofoid and White) Chitwood and *Fusarium udum* Butler are deleterious parasites of pigeon pea *Cajanus cajan* (L.) Millsp. These pathogens together are highly destructive and cause a wilt disease complex which is a major constraint for the successful cultivation of pigeon pea in India (Hasan, 1984; Siddiqui and Mahmood, 1996, 1999a).

The effects of *Pseudomonas* spp. in plant growth promotion have been observed (Lemanceau, 1992). The beneficial effects of these bacteria have been attributed to their ability to promote plant growth and to protect the plant against pathogenic micro organisms. Production of indole acetic acid (IAA) by *Pseudomonas* and its role in the development of root system is also evidenced (Patten and Glick, 2002). IAA may promote directly root growth by stimulating plant cell elongation or cell division or indirectly by influencing bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. ACC is the direct precursor of ethylene an inhibitor of root growth (Jacobson et al., 1994). The fluorescent Pseudomonads have been applied successfully to suppress *Fusarium* wilts of various plant species (Lemanceau and Alabouvette, 1993). For many Pseudomonads, production of metabolites such as antibiotics, siderophores and hydrogen cyanide (HCN) is the primary mechanism of biocontrol (Weller and Thomashow, 1993). Siderophores production during iron limited condition is responsible for the antagonism of *Pseudomonas aeruginosa* against *Pythium* spp. and root rot of many crops (Buyens et al.,

*Corresponding author. E-mail: zaki_63@yahoo.co.in.

1996; Charest et al., 2005). Many strains of Pseudomonads can indirectly protect the plants by inducing systemic resistance against various pests and diseases (Ramamoorthy et al., 2001; Van Loon et al., 1998; Zehnder et al., 2001).

PGPR can prevent the proliferation of fungal and other pathogens by producing siderophores that bind most of the Fe III in the area around the plant root. The resulting lack of iron prevents pathogens from proliferating in this immediate vicinity. The PGPR out-compete the pathogens for available iron, thus causing death of the latter. Similarly, cyanide is a secondary metabolite produced by gram negative bacteria (Askeland and Morrison, 1983). Hydrogen cyanide (HCN) and CO_2 are formed from glycine and catalyzed by HCN synthase (Castric, 1994). HCN productions by strains of *Pseudomonas* suppress diseases while mutant strain defective in synthesis of HCN lost the ability to protect plants from diseases (Sacherer et al., 1994; Voisard et al., 1989). Moreover, better availability of endogenous free IAA in the plant and a quicker defense response when infected by the fungus was reported (Fernández-Falcón et al., 2003). Over-expression of IAA according to Beckman's models (1987, 1990, 2000) could act positively on other defense factors of the host plant such as the phenolic infusion, enzyme synthesis such as glucanase, gel formation, phytoalexin synthesis and tylose formation, which may result in a significant increase of resistance of plants to pathogens.

In the present study fluorescent Pseudomonads were isolated from pathogen suppressive soils of pigeon pea fields. These isolates were tested for their hatching effect on *Meloidogyne incognita*, penetration of *M*. *incognita* and *H*. *cajani* and for the production of siderophores, hydrogen cyanide, IAA and solubilization of phosphate. Effects of these isolates on seedling growth, root colonization, growth of pigeon pea and on the reproduction of *M*. *incognita* and *Heterodera cajani* and antifungal activity against *Fusarium udum* were also studied.

MATERIALS AND METHODS

Two hundred soil and root samples of pigeon pea were collected from Aligarh district of Uttar Pradesh, India. The root samples were examined for the presence of *M. incognita*, *H. cajani* and *F. udum*. Population of *M*. *incognita* and *H*. *cajani* were also estimated in the samples (Southey, 1986). Forty samples were selected for the isolation of fluorescent Pseudomonads. Twenty one isolates of fluorescent Pseudomonads were isolated and identified using Bergey's Manual of Determinative Bacteriology (Hort et al., 1994). Bacterial colonies identified as fluorescent Pseudomonads were picked and pure culture of each isolates were maintained separately.

Effect of *Pseudomonas* on the hatching of *M. incognita*

The effects of 21 *Pseudomonas* isolates were tested on the hatching of root-knot nematode *M*. *incognita* in small Petri dishes of 9 cm diameter at 30°C. Twenty egg masses of similar size were picked with sterile forceps from the roots of pigeon pea and placed in 20 ml suspension (HiMedia Laboratories, Mumbai, India) of each *Pseudomonas* isolates (72 h old bacterial inoculum) separately for hatching. 1 ml of bacterial suspension contained 1.5×10^7 cfu/ml. For control, 20 egg-masses were placed in 20 ml of double distilled water. Each set was replicated five times and the experiment was repeated once.

Effect of *Pseudomonas* on the penetration of *M. incognita* and *H. cajani*

Penetration of *H*. *cajani* and *M*. *incognita* into pigeon pea roots was monitored in the soils preinoculated with 10 ml suspensions of different *Pseudomonas* isolates and uninoculated controls at 30°C (Table 1A). For observation of nematode penetration, 2 seeds were sown in ice-cream cups with 100 g steam sterilized sandy loam soil. One week after germination thinning was done to maintain single seedling per pot. Each seedling was inoculated with 50 s stage juveniles of *H*. *cajani* / *M*. *incognita* by exposing the roots carefully and soil was replaced. Each treatment was replicated five times. For observation, roots were taken after 20 days of inoculation. It was washed with sterile distilled water and stained with cotton blue lacto-phenol. Roots were cut into small pieces and observed under a stereo microscope and penetration by the both the nematodes was counted separately in different roots. In the control, the penetration of nematodes was observed by inoculation with 10 ml distilled water in place of bacterial suspension.

Root colonization by *Pseudomonas*

Root colonization by different isolates of *Pseudomonas* was observed to screen the effective strains. Pigeon pea roots inoculated with 72 h old culture of *Pseudomonas* isolates were collected one month after sowing. Surface sterilized 1 g roots were crushed in sterile normal saline solution (NSS) and 0.1 ml serially diluted extracts were plated on nutrient agar plates and incubated at 37°C for 24 h. The plates were placed on a Quebec colony counter for counting the bacterial colonies. Colonies falling within 30-300 range on Petri plate were selected to obtain bacterial colony number (Sharma, 2001) and represented as colony forming units (CFU) per g of root.

Effects of *Pseudomonas* isolates on the growth of seedling

Effect of *Pseudomonas* isolates (Table 1B) on growth of seedlings of pigeon pea was evaluated by the method of Shende et al. (1977) and Elliot and Lynch (1984) with slight modifications. The seeds were surface sterilized in 0.1% sodium hypochlorite (NaOCl) solution for 3 min and rinsed twice with distilled water. The bacterial cultures of each isolate were inoculated into 250 ml flasks containing 100 ml nutrient broth separately and were incubated at 37 ± 2°C for 48 h having 1.5 x 10^7 cell/ml. The seeds were dipped into the nutrient broth bacterial culture for 10 min and placed in the Petri plates of 12 cm diameter containing soft agar medium (0.8% sterile agar). These Petri plate were incubated at 32 ± 2°C for 8 days. In control treatments, the seeds were treated with sterile medium alone. The lengths of seedlings (both shoot and root) were recorded. Each set was replicated five times and the experiment was repeated once.

Hydrogen cyanide (HCN) production

Production of HCN was determined by the modified method of

Table 1A. Effect of *Pseudomonas* isolates on hatching of *M. incognita*, penetration of *M*. *incognita* and *H. cajani* in the roots and antifungal activity.

***Pseudomonas* isolates**	**J2 of *M. incognita* hatched**	**J2 of *M. incognita* per root**	**J2 of *H. cajani* per root**	**Antifungal activity shown as + (positive) and – (negative).**
Control	414	30	36	
P 701	266	20	22	-
P 703	146	11	13	-
P 704`	260	19	21	-
Pf 705	108	8	11	+
P 708	296	22	24	-
P 709	190	14	15	-
P 710	112	8	10	+
Pa 711	170	12	14	-
P 712	168	12	14	-
Pf 716	272	20	22	-
P 717	178	13	16	-
Pf 718	86	6	9	+
Pf 719	64	6	8	+
P 725	116	8	10	+
P 726	220	16	17	-
Pf 736	52	4	5	+
Pa 737	26	2	3	+
Pf 740	72	5	7	+
Pf 741	214	16	18	-
P 742	106	14	9	-
P 743	124	9	11	-
C.D. p = 0.05	7	4	5	-

Table 1b. Effect of *Pseudomonas* isolates on seedling growth and root colonization of pigeon pea (*Cajanus cajan*), phosphate solubilization, IAA and HCN production by these isolates. HCN production is shown on the basis of colour development on filter paper, i.e. a+ = Reddish brown (high); b+ = brown (moderate); c+ = light brown (low)

***Pseudomonas* isolates**	**Seedling growth (cm)**	**Phosphate solubilization (µg/mL)**	**IAA production (µg/mL)**	**HCN production**	**Colony forming units(CFU) per g of root**
Control	3.22	---	----	--	
P 701	4.98	51.0	5.75	C+	1.2×10^4
P 703	8.58	35.5	5.50	C+	1.8×10^4
P 704`	9.46	15.5	7.50	C+	1.7×10^4
Pf 705	10.04	20.0	7.25	B+	1.6×10^4
P 708	14.23	18.5	10.75	A+	1.3×10^4
P 709	9.44	17.5	5.00	C+	1.4×10^4
P 710	11.74	33.0	9.25	C+	1.8×10^4
Pa 711	13.64	24.0	11.75	A+	1.4×10^4
P 712	7.32	42.5	8.25	B+	1.6×10^4
Pf 716	10.08	57.5	8.50	B+	1.8×10^4
P 717	6.24	35.0	5.25	B+	1.9×10^4
Pf 718	5.94	29.5	5.50	B+	1.7×10^4

Table 1b. Contd.

Pf 719	6.64	31.5	6.75	C+	1.9×10^4
P 725	4.60	37.0	8.50	C+	1.8×10^4
P 726	4.80	37.0	8.50	B+	1.6×10^4
Pf 736	13.22	55.5	10.25	B+	2.1×10^4
Pa 737	8.16	49.5	5.50	B+	2.3×10^4
Pf 740	10.58	46.0	8.00	B+	2.0×10^4
Pf 741	10.38	33.0	8.75	B+	1.7×10^4
P 742	12.36	35.5	14.00	A+	1.6×10^4
P 743	8.42	33.5	7.25	B+	1.7×10^4
C.D. p = 0.05	0.76	1.9	0.52	-	-

Miller and Higgins (1970). *Pseudomonas* isolates were inoculated into 250 ml flasks containing nutrient broth and were incubated at 32 ± 1ºC. These isolates were separately streaked on nutrient agar medium supplemented with 4.4 g glycine/L with simultaneous addition of filter paper soaked in 0.5% picric acids in 1% $Na_2 CO_3$ in the upper lid of Petri plates (9 cm diameter) and the Petri plates were sealed with Parafilm. After incubation for 2 - 3 days at 32 ± 1ºC, change in colour was observed. Change in colour from yellow to light brown (low = c+), brown (moderate = b+) or reddish brown (strong = a+) showed the presence of HCN production.

Indole acetic acid (IAA) production

The production of IAA by *Pseudomonas* isolates was determined by the method of Gupta et al. (1999) with slight modifications. The cultures of bacterial isolates were grown on nutrient broth supplemented with tryptophane (5 mg/ml). 5 ml of each bacterial culture were centrifuged at 7,000 rpm (2191 x g) for 15 min at room temperature. The supernatant was collected and finally passed through the millipore filter of 0.2 µ pore size. Two ml of supernatant were mixed with two drops of o- phosphoric acid and 4 ml of freshly prepared Solawaski's reagent (50 ml of 35% perchloric acid, 1 ml 0.5% $FeCl_3$) . The development of pink colour showed the production of IAA. Absorbance was read by spectrophotometer at 530 nm. The level of IAA produced was estimated by a standard IAA graph and expressed as µg per ml.

Phosphate solubilization

Chlorostannous acid was prepared by dissolving 2.5 g of $SnCl_2.2H_20$ in 10 ml of concentrated HCl and heated as described (Saxena et al., 2002) . Volume was made up to 100 ml with distilled water. Similarly 15.0 g ammonium molybdate was dissolved in 400 ml warm distilled water. Later, 342 ml of 12 N HCl was added and allowed to cool. Volume of Chromic acid was made 1 L with distilled water. Pikovskaya broth was prepared (Pikovskaya, 1948), inoculated with *Pseudomonas* isolates separately and incubated at 28 - 30°C on a shaker for 3 - 4 days. Test bacterium was centrifuged; 1 ml aliquot from supernatant was taken and mixed with 10 ml ammonium molybdate. After shaking, it was diluted with 0.25 ml chlorostannous acid and final volume was made up to 50 ml. Intensity of blue colour was read at 600 nm. Standard curve was prepared with KH_2P0_4 to find out the amount of solubilized phosphorus in µg per ml.

Effect of *Pseudomonas* isolates on *Fusarium udum*

To observe the antifungal activity of the *Pseudomonas* isolates, *F. udum* was inoculated on the nutrient agar plates with *Pseudomonas* isolate on the same plate in dual inoculation. *F. udum* was also inoculated on nutrient agar plates singly. Plates were incubated for a week at 25°C. Growth of fungus was recorded on plates inoculated singly and also in dual inoculation. Inhibition in the growth of fungus was recorded (Table 1A).

Effect of *Pseudomonas* isolates on the growth of pigeon pea and on the wilt disease complex

Preparation of sterilization of soil mixture

Sandy loam soil (pH 7.2) was collected from a field at the Department of Botany, A.M.U., Aligarh, India, and passed through a 10 mesh sieve. Clay pots (15 cm diameter) were each filled with 1 kg of the soil. 200 ml water was poured into each pot to just wet the soil surface before sterilization at 20 pound pressure (137.9 kPa) for 20 min. Sterilized pots were allowed to cool at room temperature before use.

Raising and maintenance of test plant

Pigeon pea seeds (cv. UPAAS-120) were surface sterilized with 0.1% sodium hypochlorite (NaOCl) for 2 min and rinsed three times with sterile water. Four seeds were sown per pots and thinned to one seedling per pot one week after germination. Seedlings were subjected to the treatments listed in Tables 2 - 5. Un-inoculated plants served as a control and plants were kept in a glass house at 35 ± 5°C. Pots were arranged in a randomized block design and each treatment was replicated five times. Pots were watered as needed and the experiment was terminated after 90 days of inoculation. The experiment was repeated once.

Preparation of nematodes and fungal inocula

Large numbers of *M. incognita* egg masses were manually picked with sterile forceps from heavily infected *Solanum melongena* L. roots. These egg masses were washed in distilled water and then placed in 10 cm diameter 15 coarse sieves containing crossed layers of tissue paper placed in Petri dishes containing water. The emerged juveniles were collected at 24 h interval and subsequently fresh water was added to the Petri plates. The concentration of second stage juveniles of M. *incognita* in the water suspension was adjusted so that each mL contained 200 ± 5 nematodes. 5 ml of this suspension (1000 freshly hatched juveniles) was added around each seedling.

Table 2. Effects of four isolates of *Pseudomonas* on growth of pigeon pea (*C. cajan*) when pathogens causing the wilt disease complex were present singly under pot condition. Wilting index is based on percent necrosis in xylem; 0 = no disease; 5 = severe wilting.

Treatments		Plant dry weight (g)	No. of nodules per root system	Cysts per root system	Galls per root system	Wilting index
Control	Control	25.42	9	-	-	-
	H. cajani	18.84	5	105	-	-
	M. incognita	19.26	4	-	109	-
	F. udum	17.74	5	-	-	3
Pa737	Control	26.06	9	-	-	-
	H. cajani	21.28	4	66	-	-
	M. incognita	22.22	5	-	74	-
	F. udum	19.06	6	-	-	3
Pf736	Control	27.65	8	-	-	-
	H. cajani	21.94	6	52	-	-
	M. incognita	23.72	4	-	56	-
	F. udum	20.26	5	-	-	2
Pf718	Control	26.15	11	-	-	-
	H. cajani	20.92	9	70	-	-
	M. incognita	21.80	8	-	76	-
	F. udum	18.90	7	-	-	2
Pf719	Control	26.02	11	-	-	-
	H. cajani	20.84	7	77	-	-
	M. incognita	21.36	4	-	82	-
	F. udum	18.72	5	-	-	3
C.D. p = 0.05		0.42	3	4	6	-

Table 3. Effect of four isolates of *Pseudomonas* on growth and wilt disease complex of pigeon pea (*C. cajan*) under pot condition. Pathogens were inoculated in combination where H = *Heterodera cajani*; M = *M. incognita*; F = *F. udum*.Wilting index is based on percent necrosis in xylem; 0 = no disease; 5 = severe wilting.

Treatments		Plant dry weight (g)	No. of nodules per root system	Cysts per root system	Galls per root system	Wilting index
Control	C	25.42	9	-	-	-
	H+M	14.06	3	94	94	-
	H+F	12.37	3	85	-	4
	M+F	13.54	2	-	88	4
	H+M+F	10.26	0	78	85	5
Pa737	C	26.06	9	-	-	-
	H+M	18.09	4	61	64	-
	H+F	15.40	3	57	-	4
	M+F	15.84	2	-	66	4
	H+M+F	14.06	4	54	57	4
Pf736	C	27.65	8	-	-	-
	H+M	18.98	3	47	52	-
	H+F	16.36	2	43	-	3
	M+F	17.42	3	-	45	3
	H+M+F	15.80	4	40	41	4

Table 3. contd.

Pf718	C	26.15	11	-	-	-
	H+M	17.45	5	67	72	-
	H+F	14.64	3	64	-	3
	M+F	15.46	2	-	69	3
	H+M+F	13.48	2	59	64	4
Pf719	C	26.02	11	-	-	-
	H+M	15.80	7	72	78	-
	H+F	14.74	4	68	-	4
	M+F	15.36	3	-	74	4
	H+M+F	13.26	2	62	65	5
C.D. p = 0.05		0.39	3	5	4	-

For the inoculum of *H. cajani* , soil and root samples were collected from a pigeon pea field. Cysts were also extracted from soil using a 100 mesh sieve. The catch of sieve was filtered through a paper filter, observed under stereomicroscope and cysts were collected with a camel hair brush No.1. Collected cysts were placed for hatching in pigeon pea root exudates at 30°C for 7days. Second stage juveniles were collected after every 24 h and inoculated at the rate of 500 juveniles per plant.

F. udum was isolated from infected pigeon pea roots and maintained on potato dextrose agar (PDA) . Inoculum of this fungus was prepared by culturing the isolate in Richards liquid medium (Riker and Riker, 1936) for 15 days at 25°C. Mycelium was collected on blotting sheets and excess of water and nutrients were removed by pressing it between the two folds of the blotting sheets. 100 g of mycelium were macerated in 1 L distilled water and 10 ml of this suspension containing 1 g of the fungus were poured around the roots.

Bacterial inoculum

Nutrient agar plates were prepared by pouring sterilize nutrient agar in Petri plates. Single colony of *Pseudomonas* isolates was inoculated into each nutrient broth flask and incubated at 32 ± 1°C for 72 h. 1 ml contains about 1.5 x 10^7 CFU/ml. 10 mL of this suspension was inoculated into each pot around the pigeon pea seedling.

Inoculation technique

For inoculation of nematodes, fungus and bacterial isolates, soil around the roots was carefully removed aside without damaging the roots. The inoculum suspensions were poured around the roots and the soil was replaced. In un-inoculated control, water was added in equal volume to the inoculum suspension. Four different tests were conducted each containing a set of treatments (Tables 2 - 5). In the first test there were twenty treatments comprising of four treatments with *Pseudomonas* isolates and a control was maintained for each pathogen (Table 2). The isolates Pf718, Pf719, Pf736, and Pa737 were used. It was tested against the *H. cajani* (H), *M. incognita* (M) and *F. udum* (F). Each treatment was replicated five times and there were 100 pots in total (20 treatments x 5 replicated pots) . In a second experiment there were 25 treatments, comprising four *Pseudomonas* isolates (Control with out *Pseudomonas* isolate), Pf718, Pf719, Pf736 and Pa737) (Table 3) of the mentioned pathogen combinations (no pathogen, H + M; H + F; M + F and H + M + F) and their controls, each replicated five times for a total of 125 pots (25 treatments x 5 replicates). In a third experiment, there were 24 treatments of *Pseudomonas* and *Rhizobium* (Table 4) (No isolate; *Rhizobium*; *Rhizobium* + Pf718; *Rhizobium* + Pf719; *Rhizobium* + Pf736 and *Rhizobium* + Pa737) each tested with four combinations of pathogens [control; *H. cajani* (H); *M. incognita* (M); *F. udum* (F)] with five replicate pots per test combination (24 x 5 = 120 pots). Finally, in the fourth experiment there were 30 treat-ments of *Pseudomonas* and *Rhizobium* (Table 5) (No isolate; *Rhizobium*; *Rhizobium* + Pf718; *Rhizobium* + Pf719; *Rhizobium* + Pf736 and *Rhizobium* + Pa737) each tested with four combination of pathogens (Control; H + M; H + F; M + F and H + M + F) with 5 replicate pots per test combination (30 x 5 = 150 pots). The experiment was repeated once.

Observations

In the green house experiments, plants were uprooted after 90 days of inoculation. Root systems were gently rinsed and plants were cut above the base of the root emergence zone, and the length of shoots and roots were recorded in cm from the cut end to the top of the first leaf and longest root respectively. Excess water was removed by blotting before weighing the shoots and roots separately. The shoots were incubated at 60°C for 2 - 3 days and the dry weight was recorded. Number of cysts per root system, galls per root system and wilting index were recorded. Wilting index was recorded by scoring the disease severity on 0 - 5 scale. Index of disease was determined on the basis of percentage necrosis in the xylem, where 0 = No disease; 1 = very mild wilting; 2 = mild wilting; 3 = moderate wilting; 4 = moderately high wilting; and 5 = Severe wilting (Siddiqui and Mahmood, 1999b).

Siderophores production

Production of siderophores was estimated qualitatively on Chrome Azurol S (CAS) agar medium (Schwyn and Neilands, 1987). For preparation, 1 L of blue agar 60.5 mg CAS was dissolved into 50 ml water mixed with 10 ml Fe III solution (1 mM $FeCl_3.6H_2O$, 10 ml HCl). Under stirring this solution was slowly added to 72.9 mg HDTMA dissolved in 40 ml distilled water. The resultant dark blue liquid was autoclaved. Also, autoclaved mixture of 750 ml H_2O, 100 ml MM9 salts, 15 g agar, 30.24 g pipes (Piperazine-1,4- bis(2-ethanesulfonic acid) was added into it. Later, NaOH solution (6 M) was added to raise the pH up to 6.8 and the solution was poured into Petri plates (9 cm diameter). The active culture of the *Pseu-*

Table 4. Effect of four isolates of *Pseudomonas* and *Rhizobium* on growth of pigeon pea (*C. cajan*) when pathogens causing the wilt disease complex were present singly under pot conditions. Wilting index is based on percent necrosis in xylem; 0 = no disease; 5 = severe wilting.

Treatments		Plant dry weight (g)	No. of nodules per root system	Cysts per root system	Galls per root system	Wilting index
Control	Control	25.42	9	9	-	-
	H. cajani	18.84	5	5	-	-
	M. incognita	19.26	4	4	109	-
	F. udum	17.74	5	5	-	3
Rhizobium (R)	Control	26.12	54	54	-	-
	H. cajani	19.04	27	27	-	-
	M. incognita	19.85	32	32	98	-
	F. udum	18.08	22	22	-	2
R+Pa737	Control	26.90	57	57	-	-
	H. cajani	22.97	35	35	-	-
	M. incognita	23.06	42	42	65	-
	F. udum	20.88	31	31	-	2
R+Pf736	Control	27.85	54	54	-	-
	H. cajani	23.62	42	42	-	-
	M. incognita	23.81	46	46	49	-
	F. udum	20.68	38	38	-	1
R+Pf718	Control	27.22	52	52	-	-
	H. cajani	21.89	37	37	-	-
	M. incognita	22.70	42	42	73	-
	F. udum	20.05	31	31	-	1
R+Pf719	Control	27.30	51	51	-	-
	H. cajani	21.85	31	31	-	-
	M. incognita	22.42	36	36	75	-
	F. udum	19.38	22	22	-	2
C.D. p = 0.05		0.49	5	3	5	-

domonas isolates were spotted on the dark blue agar plates and these plates were kept into incubator for 24 - 48 h at 32 ± 1°C. Appearance of dark orange zone against the dark blue agar plates indicates the siderophore production

Statistical analysis

The entire data set was analysed as a single two factor experiment (pathogens x bacterial isolates) by the method of Dospekhov (1984). Critical differences (C.D.) were calculated at p = 0.05 and Duncan's multiple range test was employed to compare averages of the treatments.

RESULTS

Identification of the *Pseudomonas* isolates

Two hundred soil and root samples were collected from different localities of Aligarh. Twenty one isolates of rhizobacteria belonging to *Pseudomonas* were isolated from *M*. *incognita* suppressive soils of pigeon pea fields. After gram staining *Pseudomonas* identification were confirmed by growing on King's B medium. Green fluorescence of *Pseudomonas* spp. was very clear on King's B medium. Characterization tests for the identification of *Pseudomonas* showed that out of the twenty one isolates, six isolates were positive for levan formation, gelatin liquefaction, growth at 4°C and catalase test while negative for starch hydrolysis and growth at 41°C. Comparing these characteristics with Bergey's Manual of Determinative Bacteriology, the seven isolates were identified as *P*. *fluorescens* and named as Pf705, Pf716, Pf718, Pf719, Pf736, Pf740 and Pf741 while two isolates (Pa711 and Pa737) were positive for growth at 41°C, gelatin liquefaction, citrate utilization while negative for levan formation and growth at 4°C, thus identified as *P. aeruginosa*. The remaining twelve isolates may belong to some other species of *Pseudomonas* and need further characterization.

Effect of the isolates on the nematodes

The isolates Pa737 caused greater inhibitory effect (93%) on the hatching of *M*. *incognita* followed by Pf736 (87%), Pf719 (84%), and Pf718 (79%). Isolate P708 was the

least effective (28%) in the inhibition of hatching of *M*. *incognita* (Table 1A). Similarly, isolate Pa737 caused greater adverse effect (93 and 91%) on the penetration of *M*. *incognita* and *H*. *cajani* in the roots of pigeon pea, followed by Pf736, Pf719, and Pf718. Again, isolate P708 was least effective in inhibiting the penetration of both the nematodes in the roots.

Antifungal activity of the isolates

All the 21 isolates of *Pseudomonas* were tested for their antifungal activity against *F. udum* in dual inoculation test. Only 8 isolates of *Pseudomonas* (P704, P710, Pf718, Pf719, P725, Pf738, Pa737 and Pf740) showed antifungal activity, while the remaining 13 isolates had no antifungal effect against *F*. *udum* (Table 1A).

Root colonization by the *Pseudomonas* isolates

All the 21 isolates of *Pseudomonas* colonized pigeon pea roots but root colonization by different isolates was variable. Maximum colonization of pigeon pea roots was caused by isolate Pa737 followed by Pf736 and Pf740. However, isolates P701 caused minimum colonization of pigeon pea roots. The root colonization by different *Pseudomonas* isolates was between 2.3×10^4 to 1.2×10^4 (Table 1B).

Effect of *Pseudomonas* isolates on the growth of pigeon pea seedling

All the 21 isolates of *Pseudomonas* caused a significant increase in pigeon pea seedling growth when seeds were treated with isolates of *Pseudomonas* were grown (Table 1B) . Isolate P708 caused maximum increase (342%) in the growth of seedlings followed by Pa711 (324%), Pf736 (309%), P742 (284%) and P710 (265%). Isolate P725 caused minimum increase (43%) on seedling growth.

Phosphate solubilization by *Pseudomonas* isolates

All the 21 isolates caused solubilization of phosphate (Table 1B). Isolate Pf716 caused 57.5 g/mL phosphate solubilization followed by Pf736 (55.5 g/mL), P701 (51.0 g/mL) and Pa737 (49.5 g/mL). Minimum solubilization of phosphate (15.5 g/mL) was caused by isolate P704.

IAA production

Isolate P742 had maximum production of IAA followed by Pa711, P708, and Pf736 (Table 1B) . Isolate P742 produced 14 g/mL while 5 g /mL was least IAA produced by isolate P709.

HCN production

Out of twenty one isolates, three isolates namely, P708 Pa711 and P742 had the ability to produce maximum quantity of HCN compared to other isolates in the present investigation (filter paper changed into reddish color) (Table 1B). Eleven isolates, namely Pf705, P712, Pf716, P717, Pf718, P726, Pf736, Pa737, Pf740, Pf741, and P743 developed brown color and, therefore, had moderate production of HCN. The remaining seven isolates showed low production of HCN by as they developed light brown color.

Effect on plant growth

Inoculation with *M*. *incognita*, *H*. *cajani* and *F*. *udum* singly caused a significant reduction in plant growth over uninoculated control (Table 2). Inoculation of plants with isolates of *Pseudomonas* caused a significant increase on plant growth both inoculated with pathogen and also of uninoculated control. Increase in the growth caused by *Pseudomonas* isolates in plants with pathogens was greater compared to plants with out pathogens. Isolate Pf736 caused the greatest increase in plant growth followed by Pa737, Pf718, *Rhizobium* nodulation was poor in all the plants inoculated with pathogens and also in unionculated control. Inoculation of plants with *Pseudomonas* isolates had no effect on nodulation. Instead, *Pseudomonas* isolates had adverse effect on nematode multiplication, with isolate Pf736 causing greater reduction in nematode multiplication followed by Pa737, Pf718, and Pf719 (Table 2).

Simultaneous inoculation with *M*. *incognita*, *H*. *cajani*, and *F*. *udum* caused significant reduction in plant growth over uninoculated control (Table 3). Inoculation of *H*. *cajani* with *F*. *udum* resulted in greater reduction in plant growth followed by inoculation of *M*. *incognita* with *F*. *udum*. Inoculation of *H*. *cajani* with *M*. *incognita* caused less reduction in the plant growth compared to plants inoculated with *F*. *udum* plus *M*. incognita / *H*. *cajani*. The greatest reduction in plant growth was observed when all the three pathogens were inoculated simultaneously. Isolates of *Pseudomonas* caused significant increase in the growth of plant both inoculated with pathogens and also of uninoculated control. Increase in the growth caused by *Pseudomonas* isolates in plants with pathogens was greater compared to plants without pathogens. Greater growth increase was observed in plants inoculated with isolate Pf736, followed by Pa737, Pf718, and Pf719 (Table 3). Again *Rhizobium* nodulation was poor in all the plants inoculated with pathogens and in unionculated control. *Pseudomonas* isolates had no effect on nodulation but they had an adverse effect on nematode multiplication. Isolate Pf736 caused the greatest reduction in nematode multiplication followed by Pa737, Pf718, and Pf719.

Inoculation pathogens singly caused a significant reduction in plant growth over uninoculated control (Table 4). Inoculation of plants with *Rhizobium* or isolates of *Pseudomonas* alone caused a significant increase in the growth of plant both inoculated with pathogen and also of uninoculated control. *Rhizobium* caused less increase in plant growth than *Pseudomonas* isolates. However, increase in the growth caused by *Pseudomonas* isolates/*Rhizobium* in plants inoculated with pathogens was greater compared to plants without patho-gens. Isolate Pf736 caused the greatest increase in plant growth followed by Pa737, Pf718, and Pf719. Combined use of *Pseudomonas* isolate with *Rhizobium* was better in increasing plant growth than the use of either one. The combined use of Pf736 with *Rhizobium* caused greater increase in plant growth than any of the other *Rhizobium*/*Pseudomonas* combinations (Table 4).

Nodulation was high in plants inoculated with *Rhizobium*. Inoculation of pathogens singly in plants with *Rhizobium* had adverse effect on nodulation. Inoculation of *Pseudomonas* isolates with *Rhizobium* in plants with pathogens increased nodulation compared to plant inoculated with pathogens and *Rhizobium*. *Pseudomonas* isolates/*Rhizobium* had adverse effect on number of galls and cysts per plant. Isolate Pf736 caused greater reduction in the number of galls and cysts per plant followed by Pa737, Pf718, and Pf719. Maximum reduction in number of galls and cysts per plant was observed when Pf736 was used along with *Rhizobium* (Table 4). Simultaneous inoculation of two or all the three pathogens caused a significant reduction in plant growth over un-inoculated control (Tables 3 and 5). Greatest reduction in plant growth was observed when all the three pathogens were inoculated simultaneously. Isolates of *Pseudomonas*/*Rhizobium* caused a significant increase in the plant growth where pathogens were inoculated simultaneously and also in uninoculated control. Increase in the growth caused by *Pseudomonas* isolates in plants with pathogens was greater compared to plants inoculated with *Rhizobium*. Isolate Pf736 caused the greatest increase in plant growth followed by Pa737, Pf718, and Pf719. Use of *Rhizobium* with Pf736 caused maximum increase in plant growth compared to plants inoculated with *Rhizobium* plus other isolates of *Pseudomonas* (Tables 3 and 5). Nodulation was poor in all the plants without *Rhizobium* and high in plant inoculated with *Rhizobium* (Table 5). Combined inoculation of pathogens in plants with *Rhizobium* had adverse effect on nodulation. However, inoculation of *Pseudomonas* isolates with *Rhizobium* increased nodulation. *Pseudomonas* isolates/*Rhizobium* had adverse effect on galling and cysts per plant. Isolate Pf736 caused the greatest reduction in galling and cysts per plant followed by Pa737, Pf718, and Pf719. Use of Pf736 with *Rhizobium* caused greater reduction in nematode multiplication than the use of *Rhizobium* with other isolates of *Pseudomonas* (Table 5).

Wilting index

Wilting index was found 3 when *F*. *udum* was inoculated alone (Table 2). Combined inoculation of *F*. *udum* with Pf736 or Pf718 recorded a minimum wilt index of 2 when compared to that of inocultion alone. However the wilting index was 4 when *F*. *udum* was coinoculated with root knot or cyst nematodes. But combined inoculation of *F. udum* along with the nematodes had the maximum wilt index of 5. Combined inoculation of Pf736 or Pf718 along with root knot and cyst nematode recorded a wilt index of 3 compared to combined inoculation of all pathogens. Similarly, index was reduced to 4 when plants with all the three pathogens were inoculated with Pf736 or Pf718. Inoculation of *Rhizobium* with Pf736 or Pf718 reduced wilting index to 1 in plants with *F. udum* while it was reduced to 2 in plants with fungus plus either nematode species were treated with *Rhizobium* alongwith Pf736 or Pf718 (Tables 4 and 5).

Siderophores production

Of the twenty one isolates of Pseudomonas tested, only twelve showed production of siderophores in CAS medium (Figure 1). Isolate Pf736 showed greater siderophores production followed by Pf719, Pa737 and Pf705. Eight isolates were unable to produce siderophores in CAS medium (Figure 1).

DISCUSSION

Most of the *Pseudomonas* isolates used in this study increased growth of nematode inoculated and uninoculated plants. Among the twenty isolates used, isolates Pf736 and Pa737 were best in improving plant growth and reduced galling, cyst formation, nematode multiplication and wilting index than the other isolates used. *Pseudomonas* are known to suppress diseases by inhibition of pathogens by competition of Fe (III), inhibition of pathogen by diffusible or volatile products, induction of resistance in plants and aggressive root colonization and stimulation of plant growth (Kloepper et al., 1980; Siddiqui, 2006; Weller, 1988). Isolates Pf736 and Pa737 caused greater root colonization than the other isolates. This may be why isolates Pf736 and Pa737 caused greater increase in plant growth and greater reduction in nematode multiplication than the other isolates in our study. Moreover, greater seedling growth and greater phosphate solubilization was observed with these isolates than with the other isolates. This also confirms that these isolates have greater plant growth promoting effect than caused by other isolates. *Pseudomonas* spp. Produces a wide variety of antibiotics, growth promoting hormones, siderophores, HCN and can solubilize phosphorous (Kraus and Loper, 1995; Rodriguez and Fraga,

Table 5. Effect of four isolates of *Pseudomonas* and *Rhizobium* on growth and wilt disease complex of pigeon pea (*C. cajan*) under pot conditions. Pathogens were inoculated in combination where H = *Heterodera cajani*; M = *M. incognita*; F = *F. udum*. Wilting index is based on percent necrosis in xylem; 0 = no disease; 5 = severe wilting.

Treatment		Plant dry weight (g)	No. of nodules per root system	Cysts per root system	Galls per root system	Wilting index
Control	C	25.42	9	-	-	-
	H+M	14.06	3	94	94	-
	H+F	12.37	3	85	-	4
	M+F	13.54	2	-	88	4
	H+M+F	10.26	0	78	85	5
Rhizobium (R)	C	26.12	54	-	-	-
	H+M	15.22	21	84	85	-
	H+F	13.41	16	75	-	3
	M+F	14.09	18	-	80	3
	H+M+F	11.10	8	68	77	4
R+Pa737	C	26.90	57	-	-	-
	H+M	18.10	25	57	60	-
	H+F	16.52	26	48	-	3
	M+F	17.84	28	-	53	3
	H+M+F	14.90	19	43	48	3
R+Pf736	C	27.85	54	-	-	-
	H+M	19.58	31	39	44	-
	H+F	17.66	33	34	-	2
	M+F	18.45	31	-	39	2
	H+M+F	15.69	23	31	33	3
R+Pf718	C	27.22	52	-	-	-
	H+M	17.67	26	64	67	-
	H+F	15.60	28	59	-	2
	M+F	16.64	29	-	62	2
	H+M+F	13.92	20	55	59	3
R+Pf719	C	27.30	51	-	-	-
	H+M	17.21	20	68	69	-
	H+F	15.29	21	61	-	3
	M+F	16.52	19	-	68	3
	H+M+F	13.60	13	57	61	4
C.D. p = 0.05		0.46	4	4	6	-

1999; Seong and Shin, 1996) . Most of the isolates of *Pseudomonas* we tested were successful in the biocontrol of wilt disease complex of pigeon pea. Isolates Pf736 and Pa737 were found best in improving plant growth and reducing galling, cyst formation and nematode multiplication when compared with other isolates. Isolate Pf736 and Pa737 produced greater amount of siderophores, HCN, IAA and colonized the roots greater than other isolates used. The plant growth promotion ability of fluorescent *Pseudomonas* is a function of good root colonization and production of growth hormones as reported earlier (O'Sullivan and O'Gara, 1992; Weller, 1988).

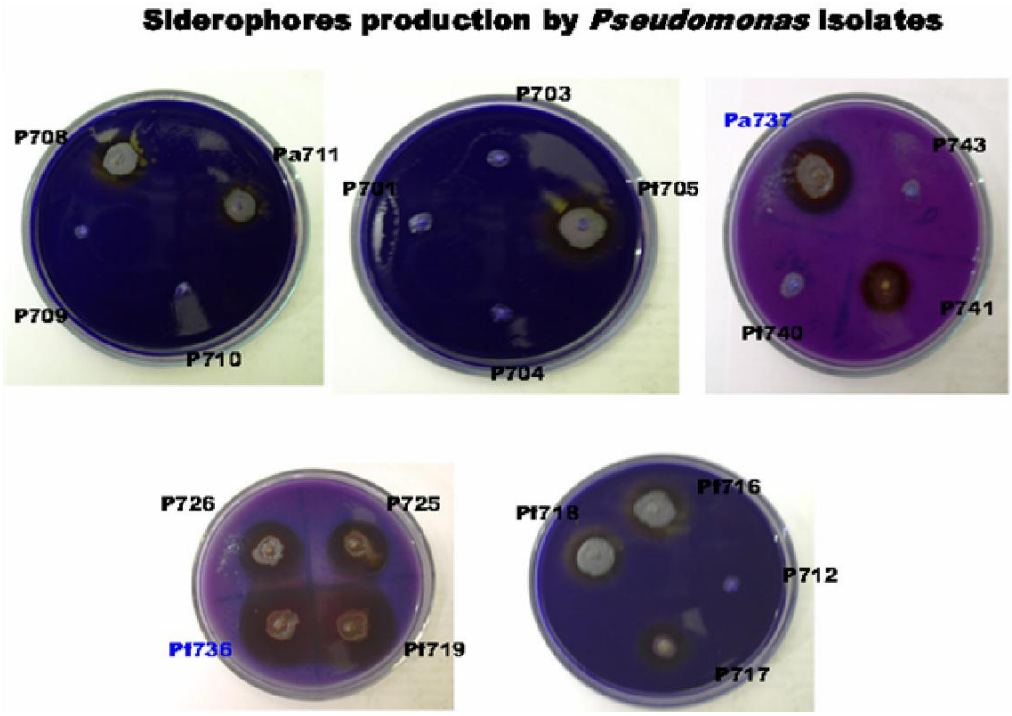

Figure 1. Of the 20 isolates of *Pseudomonas*, 12 isolates showed production of siderophores in CAS medium. Isolate Pf736 showed greater siderophores production and isolate P717 the least. Isolates P701, P703, P704, P709, P710, Pf740 and P743 did not produce siderophores in CAS medium.

Moreover, various secondary metabolites secreted by *Pseudomonas* spp., including HCN and siderophores, have been found to be inhibitory against different phytopathogens (Bagnasco et al., 1998; Siddiqui, 2006). Good inhibition of nematodes in rhizospheric condition was achieved by isolates Pf736 and Pa737 than other isolates used because these isolates were able to produce more HCN and siderophores which are reported to antagonize plant pathogens.

The populations of both nematodes and wilting index were also reduced in *Rhzobium* inoculated and non inoculated plants. Root nodules induced by *Rhizobium* fix atmospheric nitrogen and are reported to produce toxic metabolites inhibitory to many plant pathogens (Haque and Ghaffar, 1993) . Barker and Huisingh (1970) observed necrosis in nodular tissues following invasion by nematodes, this may account in part for reduced nematode development. *Rhizobium* secretes rhizobitoxine (Chakraborty and Purkayastha, 1984) while *Rhizobium leguminosarum* (Frank) Frank is reported to produce increased levels of phytoalexin (4-hydroxy-2, 3, 9, trimethoxy pterocarpan) in pea (Chakraborty and Chakraborty, 1989). Roslycky (1967) reported production of an antibiotic bacteriocin by rhizobia. All this suggests that application of rhizobia which increase nitrogen content and plant growth can also reduce the adverse effect of plant pathogens (Siddiqui and Mahmood, 1995).

The present study demonstrate that a root nodule bacterium and a plant growth promoting rhizobacterium *Pseudomonas* can coexist without exhibiting adverse effects on each other. These biocontrol agents may be used concomitantly for the biocontrol of diseases. Therefore, in future studies more detailed investigations of the relationships in various pathosystems and interactions between the microorganisms and the host plant are needed for developing biocontrol of related plant diseases.

For the practical application of these biocontrol agents in the field, inoculum of *P. fluorescens* may be mixed in the charcoal culture of *Rhizobium* and the mixture of these biocontrol agents may be used as seed treatment. This will provide protection against wilt disease complex of pigeon pea and would enhance the successful cultivation of pigeon peas under field conditions.

ACKNOWLEDGEMENTS

The authors are grateful to Department of Science and Technology, New Delhi, for the financial assistance through Project No. SP/SO/A22 /2001.

REFERENCE

Askeland RA, Morrison SM (1983). Cyanide production by *Pseudo-*

monas fluorescens and *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 45:1802-1807.
Bagnasco P, De La Fuente L, Gaultieri G, Noya F, Arias A (1998). Fluorescent *Pseudomonas* spp. as biocontrol agents against forage legume root pathogenic fungi. Soil Biol. Biochem. 10:1317-1323.
Barker KR, Huisingh D (1970). Histopathological investigations of the antagonistic interaction between *Heterodera glycines* and *Rhizobium japonicum* on soybean. Phytopathology 60:1282-1283.
Beckman CH (1987). The Nature of Wilt Diseases of Plants. American Phytopathological Society Press. St. Paul, MN, USA. 175p.
Beckman CH (1990). Host responses to the pathogen. pp. 93-105. In: R.C. Ploetz (ed.). *Fusarium* Wilt of Banana. Amer. Phytopathol. Soc. Press. St. Paul, MN, USA.
Beckman CH (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? Physiol. Mol. Plant Pathol. 57:101-110.
Buyens S, Heungens K, Poppe J, Höfte M (1996). Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl. Environ. Microbiol. 62:865-871.
Castric P (1994). Influence of oxygen on the *Pseudomonas aeruginosa* hydrogen cyanide synthase. Curr. Microbiol. 29:19-21.
Chakraborty U, Chakraborty BN (1989). Interaction of *Rhizobium leguminosarum* and *Fusarium solani* f. sp. *pisi* in pea affecting disease development and phytoalexin production. Can. J. Bot. 67:1698-1701.
Chakraborty U, Purkayastha RP (1984). Role of Rhizobitoxine in protecting soybean roots from *Macrophomina phaseolina* infection. Can. J. Microbiol. 30:285-289.
Charest MH, Beauchamp CJ, Antoun H (2005). Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and *Pythium ultimum.* FEMS Microbiol. Ecol. 52:219-227.
Dospekhov BA (1984). Field experimentation. Statistical procedures. Mir Publishers, Moscow. p. 352.
Elliot LF, Lynch JM (1984). *Pseudomonas* as a factor in the growth of winter wheat (*Triticum aestivum* L.). Soil Biol. Biochem. 16:69-71.
Fernández-Falcón M, Borges AA, Borges -Pérez A (2003). Induced resistance to *Fusarium* wilt of banana by exogenous applications of indolacetic acid. Phytoprotec. 84:149-153.
Gupta CP, Sharma A, Dubey RC, Maheshwari DK (1999). *Pseudomonas aeruginosa* as a strong antagonist of *Macrophomina phaseolina* and *Fusarium oxysporum*. Cytobiosis 99:183-189.
Haque SE, Ghaffar A (1993). Use of rhizobia in the control of root-rot disease of sunflower, okra, soybean and mungbean. Phytopath. Z. 138:157-163.
Hasan A (1984). Synergism between *Heterodera cajani* and *Fusarium udum* attacking *Cajanus cajan*. Nematol. Medit.12:159-162.
Hort JC, Kreig NR, Sneath PHA, Staley JT, William ST (1994). Bergey's Manual of Determinative Bacteriology, 9th ed. William and Wilkins. Baltimore, MD, USA. p. 244.
Jacobson CB, Pasternak JJ, Glick BR (1994). Partial purification and characterization of ACC deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. Can. J. Microbiol. 40:1019-1025.
Kloepper JW, Leong J, Teintze M, Schroth MN, (1980). Enhanced plant growth by plant growth promoting rhizobacteria. Nature 286:885-886.
Kraus J, Loper J, (1995). Characterization of genomic region required for production of antibiotic pyoluteorin by the biological control agent *Pseudomonas fluorescens* Pf-5. Appl. Environ. Microbiol. 61:849-854.
Lemanceau P (1992). Effets bénéfiques de rhizobactéries sur les plantes: exemple des *Pseudomona*s spp. fluorescents. Agronomie 12:413-437.
Lemanceau P, Alabouvette C (1993). Suppression of *Fusarium* wilts by fluorescent pseudomonas: mechanisms and applications. Bioc. Sci. Technol. 3:219-234.
Miller RL, Higgins VJ (1970). Association of cyanide with infection of birdfoot trefoil *Stemphylium loti*. Phytopathology 60:104-110.
O'Sullivan DJ, O'Gara F (1992). Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant pathogens. Microbiol. Rev. 56:662-672. supply initials
Patten CL, Glick BR (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68:3795-3801.
Pikovskaya RI (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologiya 17:362-370.
Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protec. 20:1-11.
Riker AJ, Riker R.S (1936). Introduction to Research on Plant diseases. John's Switft Co. Inc. St. Louis, Chicago, New York, Indianapolis, USA. p.177.
Rodriguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv.17:319-339.
Roslycky EB (1967). Bacteriocin production in the rhizobia bacteria. Can. J. Microbiol. 13:431.
Sacherer P, Défago G, Haas D (1994). Extracellular protease and phosholipase C are controlled by the global regulatory gene *gacA* in the biocontrol strain *Pseudomonas fluorescens* CHA0. FEMS Microbiol. Lett. 116:155-160.
Saxena AK, Lata, Annapurna K (2002). Training Manual on Plant Growth Promoting Rhizobacteria. Division of Microbiology, Indian Agricultural Research Institute. New Delhi, India. p. 38.
Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophores. Ann. Biochem. 160:47-56.
Seong KY, Shin PG (1996). Effect of siderophore on biological control of plant pathogens and promotion of plant growth by *Pseudomonas fluorescens* ps88. Agric. Chemis. Biotechnol. 39:20-24.
Sharma PD (2001). Microbiology. Rastogi and Company. Meerut, India. p. 359.
Shende ST, Apte RG, Singh T (1977). Influence of *Azotobacter* on germination of rice and cotton seeds. Curr. Sci. 46:675-676.
Siddiqui ZA (2006). PGPR: Prospective biocontrol agents of plant pathogens. pp. 111-142. In: Z.A. Siddiqui (ed.). PGPR: Biocontrol and Biofertilization. Springer. Dordrecht, The Netherlands.
Siddiqui ZA, Mahmood I (1995). Biological control of *Heterodera cajani* and *Fusarium udum* by *Bacillus subtilis*, *Brandyrhizobium japonicum* and *Glomus fasciculatum* on pigeon pea. Fundam. Appl. Nematol. 18:559-66.
Siddiqui ZA, Mahmood I (1996). Effects of *Heterodera cajani*, *Meloidogyne incognita* and *Fusarium udum* on the wilt disease complex of pigeonpea. Indian J. Nematol. 26:102-04.
Siddiqui ZA, Mahmood I (1999a). Role of bacteria in the management of plant parasitic nematodes - A Review. Bioresource Technol. 69:167-79.
Siddiqui ZA, Mahmood I (1999b). The effect of inoculations of *Heterodera cajani* and *Meloidogyne incognita* with *Fusarium udum* and *Bradyrhizobium japonicum* on the wilt disease complex of pigeonpea. Indian Phytopath. 52:66-70
Southey JF (1986). Laboratory Methods for Work with Plant and Soil Nematodes. 6th ed. Ministry of Agriculture Fisheries and Food. Reference Book No. 402, HMSO. London, UK. p. 202.
Van Loon LC, Bakker PAHM, Pieterse CMJ (1998). Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36:453-483.
Voisard C, Keel C, Haas D, Defago G (1989). Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO Journal 8:351-358.
Weller DM (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
Weller DM, Thomashow LS (1993). Use of rhizobacteria for biocontrol. Curr. Opin. Biotechnol. 4:306-311.
Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW (2001). Application of rhizobacteria for induced resistance. Eur. J. Plant Pathol. 107:39-50.