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Use of Fluorescent *Pseudomonads isolates* for the Biocontrol of Wilt Disease Complex of Pigeonpea in Green House Assay and under Pot Condition

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Abstract: Twenty isolates of fluorescent *Pseudomonads* were isolated from pathogen suppressive soils of pigeonpea fields. Thirteen isolates were identified as *Pseudomonas aeruginosa*, 5 as *P. fluorescens* while remaining 2 needs characterization *Pseudomonas* sp. Four isolates (Pf604, Pf605, Pf611 and Pa616) were considered potential for the biocontrol of the disease on the basis of antibiotics sensitivity, antifungal activity, fluorescence produced by *Pseudomonas*, inhibitory effect on the hatching and penetration of nematodes and colonization of pigeonpea roots by these isolates. Evaluation of these isolates under pot condition revealed that isolate Pf605 was the best biocontrol agent for the wilt disease complex followed by Pf604, Pf611 and Pa616. Isolate Pf605 caused greater reduction in nematode multiplication and also reduced wilt index under pot condition.

Key words: Biocontrol, pigeonpea, *Pseudomonas*, wilt disease complex

INTRODUCTION

Pigeonpea, *Cajanus cajan* (L.) Millsp., is an important pulse crop of India and is major source of protein for most of the vegetarian population. Pigeon pea is susceptible to *Heterodera cajani* Koshy, *Meloidogyne incognita* (Kofoid and White) Chitwood and *Fusarium udum* Butler. These pathogens are deleterious parasites of pigeonpea and together and cause a wilt disease complex, which is a major constraint for the successful cultivation of pigeonpea in India[1-3]. There is an urgent need to work out a suitable biocontrol of a wilt disease complex of pigeonpea.

Large numbers of rhizobacteria have been used as biocontrol agents[4,5] and *Pseudomonas* sp. are well know for their involvement in the biocontrol of several plant pathogens[6]. The selection of effective strains of particular bacteria is of prime importance for the biocontrol of plant pathogens. Isolation of bacteria from pathogen suppressive soils may increase the chances of finding effective strains[7]. To get effective isolates, isolation of bacteria should be made from the same environment in which they will be used[8]. The ability to colonize oots and resistance against antibiotics are the other parameters to screen effective strains[9]. Potential isolates found through green house assay should also be tested under pot condition for their biocontrol potential before their use in the field for the biocontrol of wilt disease complex of pigeonpea.

An attempt was made to isolate fluorescent *Pseudomonads* from pathogen suppressive soils of pigeonpea fields. These isolates were screened against antibiotics and *Fusarium udum*. Effects of these isolates on the hatching of *Meloidogyne incognita*, penetration of *M. incognita* and *Heterodera cajani* and root colonization of pigeonpea were also observed. Later these isolates were tested for their biocontrol potential in pots both in mono and multi pathogenic condition.

MATERIALS AND METHODS

Four hundred forty soil and pigeonpea root samples were collected from 22 districts of U.P., India. The root and soil samples were examined for the presence of *Meloidogyne* spp. and *Heterodera cajani* and population of these nematodes were estimated[10]. Moreover, isolation of *Fusarium udum* was made on PDA from infected roots and pure culture was maintained. Suppressive soils became apparent when wilt incidence or severity of wilt disease was lower than expected for the prevailing environment as compared to the surrounding soil. Moreover, samples of these areas were showing poor numbers of above mentioned nematodes. These samples were later selected for the isolation of fluorescent pseudomonads. Rhizobacterial isolations were made on nutrient agar and nutrient broth. Twenty isolates of fluorescent pseudomonads were isolated and identified using Bergey's manual of determinative bacteriology[11] as

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shown in Table 1. Bacterial colonies identified as fluorescent pseudomonads were picked and pure culture of each isolate was maintained separately.

Antibiotic sensitivity tests: Antibiotic sensitivity of fluorescent pseudomonads was determined by standard disc diffusion method of Baur *et al.*[12] and as adopted by Antoun *et al.*[13]. The antibiotic discs were obtained from the Hi-media laboratories Pvt. Ltd. Bombay, India. Antibiotic discs used were Ampicillin, Penicillin, Rifampicin, Ciprofloxacin, Co-Trimoxazole and Nalidixic acid (25 mc g/disc). The results are presented in the Table 2.

Effect of rhizobacteria on hatching of root-knot nematodes: Effects of 20 isolates were observed on the hatching of root-knot nematode *Meloidogyne incognita* in small petri dishes at 30°C. Twenty egg-masses of almost similar size were picked with sterilized forceps from the roots of *Solanum melongena* and placed in 20 mL suspension of each isolate separately for hatching. One milliliter of bacterial suspension contains $1.5x10^7$ bacterial cells mL^{-1}. For control, twenty egg-masses were placed in 20 mL double distilled water. Each set was replicated 5 times and experiment was repeated once. Effect of different isolates on hatching was determined as shown in Table 3.

Effect of rhizobacteria on nematodes penetration: Penetration of *H. cajani* and *M. incognita* was observed into pigeonpea roots both inoculated with rhizobacteria and uninoculated ones at 30°C. For observation of nematode penetration, seeds were sown in ice cream cups with 100 g steam sterilized soil. Ten ml of each *Pseudomonas* isolate was poured in 20 g soil and 50 seeds were wrapped in this soil. One week after germination seedlings were inoculated with 50 sec 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 out, washed with distilled and stained with cotton blue lactophenol. Roots were cut into small pieces and observed under the stereomicroscope. Penetration of nematodes was calculated and presented in Table 3.

Root colonization by rhizobacteria: Root colonization by different isolates of rhizobacteria was also observed to screen the effective strains. Pigeonpea roots inoculated with rhizobacteria were collected one month after sowing. Surface sterilized 1 g root was crushed in sterilized Normal Saline Solution (NSS) and 0.1 mL serially diluted extracts were plated on nutrient agar plates. The plates were placed on Quebec colony counter for counting of bacterial colonies. The colony falling in the range of 30-300 was selected and multiplied by reciprocal dilution factor to obtain bacterial colony[14] and represented as Colony Forming Units (CFU) per g of root (Table 3).

Effect of rhizobacteria on the fungus: To observe the antifungal activity of *Pseudomonas* isolates, *Fusarium udum* was inoculated on the nutrient agar plates with *Pseudomonas* isolate on the same plate. Inhibition in the growth of *F. udum* was observed and recorded as shown in Table 3.

Pot experiment

Preparation and sterilization of soil mixture: Sandy soil collected from a field belonging to Botany Department, A.M.U. Aligarh was passed through a 10 mesh sieve. The soil was mixed with river sand in the ratio of 4:1 (v/v) and 15 cm diameter clay pots each filled with 1 kg of the mixture. Water was poured into each pot to just wet the soil before transferring them to an autoclave for sterilization at 137.9 kPa for 20 min. Sterilized pots were allowed to cool at room temperature before use.

Raising and maintenance of the test plant: Pigeonpea (*Cajanus cajan*) seeds Cultivar UPAAS-120 were surface sterilized with 0.01% sodium hypochlorite for 2 min and rinsed three times with sterile water. Three seeds were sown in 15 cm earthen pots having steam sterilized soil. One week after germination thinning was done to maintain single plant per pot and seedlings were subjected to the treatments listed in Table 1 and 2. Uninoculated plants served as controls. Plants were kept in a greenhouse bench at a temperature ranging from 30-40°C in a factorial block design. Pots were watered as needed. Each treatment combination was replicated 5 times and experiment was terminated 90 days after inoculation.

Preparation of nematodes and fungal inoculum: Large numbers of *M. incognita* egg-masses were manually picked with sterilized forceps from heavily infected *Solanum melongena* roots on which pure culture of *M. incognita* was maintained. These egg masses were washed in distilled water and then placed in 10 cm diameter 15 coarse sieves containing crossed layers of tissue paper which were subsequently placed in petri dishes containing water just deep enough to come in contact with egg masses. The hatched juveniles were

Table 1: Characteristic tests for identification of *Pseudomonas* spp.

Biochemical test	Pa601	Pa602	Pa603	Pf604	Pf605	Pa606	Pf607	Pf608	Pa609	Pa610
Gram reaction	-	-	-	-	-	-	-	-	-	-
Gelatin liqefaction	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Growth on kings B medium	++	++	++	++	+	++	+(L)	+(L)	+	++
Growth at 4°C -	-	-	+	+	-	-	-	-	-	-
Growth at 41°C	-	-	-	-	-	-	+	+	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+
Levan formation	-	-	-	+	+	-	+	+	-	-

Biochemical test	Pf611	Pa612	Pa613	Pa614	Pa615	Pa616	P617	P618	Pa619	Pa620
Gram reaction	-	-	-	-	-	-	-	-	-	-
Gelatin liqefaction	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Growth on kings B medium	++	++	+(L)	++	++	++	++	++	+	+(VL)
Growth at 4°C +	-	-	-	-	-	-	-	-	-	-
Growth at 41°C	-	-	-	-	-	+	+	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+
Levan formation	+	-	-	-	-	-	+	+	-	-

- = test negative, + = test positive, ++ = growth very good, +(L) = growth poor, +(VL) = growth very poor

Table 2: Antibiotics sensitivity to 20 isolates of *Pseudomonas*

Isolates	Ampicillin A^{10}	Penicillin P^{10}	Rifampicin R^{30}	Ciprofloxacin Cf^{10}	Co-trimoxazole Co^{25}	Nalidixic acid Na^{30}
Pa601	R	R	14 mm	25 mm	R	R
Pa602	R	R	15 mm	28 mm	R	R
Pa603	R	R	11 mm	23 mm	R	R
Pf604	R	R	31 mm	20 mm	R	R
Pf605	R	R	13 mm	20 mm	R	R
Pa606	R	R	11 mm	21 mm	R	R
Pf607	R	R	15 mm	24 mm	R	R
Pf608	R	R	15 mm	30 mm	R	R
Pa609	R	R	18 mm	21 mm	R	R
Pa610	R	R	11 mm	23 mm	10 mm	R
Pf611	R	R	13 mm	20 mm	R	R
Pa612	R	R	16 mm	18 mm	R	R
Pa613	R	R	30 mm	10 mm	R	R
Pa614	R	R	19 mm	30 mm	R	R
Pa615	R	R	17 mm	32 mm	R	10 mm
Ps616	R	R	27 mm	20 mm	R	R
P617	R	R	13 mm	20 mm	R	R
P618	R	R	11 mm	21 mm	R	R
Pa619	R	R	17 mm	22 mm	R	R
Pa620	R	R	28 mm	20 mm	R	R

collected from the Petri plates every 24 h and 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. Five millliter this suspension (i.e., 1000 freshly hatched juveniles) was added around seedling.

For the inoculum of *Heterodera cajani*, soil and root samples were collected from pigeonpea field. Roots were examined for the cysts under stereomicroscope; cysts present on the roots were collected. Cysts were also isolated from soil through 100 mesh sieve and the catch of sieve was filtered through filter paper. Catch of filter paper was observed under stereomicroscope, cysts observed were collected through camel hair brush No.1. Cysts thus collected were placed for hatching in pigeonpea root exudates. Juveniles collected were inoculated at the rate of 500 juveniles per plant.

Fusarium udum was isolated from infected pigeonpea roots and maintained on Potato Dextrose Agar (PDA). Inoculum of this fungus was prepared by culturing the isolate in Richards liquid medium[15] 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. Hundred gram mycelium was macerated in 1 l distilled water and 10mL of this suspension containing 1 g fungus was inoculated around the roots.

Bacterial inoculum: Each isolate was cultured on nutrient agar plates and growth of each isolate was scrapped with

Table 3: Effects of 20 isolates of *Pseudomonas* on hatching of *M. incognita*, penetration of *M. incognita* and *H. cajani*, root colonization and antifungal activity

Isolates	No. of *M. incognita* hatched	No. of *M. incognita* penetrated	No of *H. cajani* penetrated	Root colonization	Antifungal activity
DDW	435	32	35	------	----------
Pa601	251	14	16	$1.6x\ 10^4$	+
Pa602	341	22	23	$1.1x\ 10^4$	+
Pa603	286	18	19	$1.4x\ 10^4$	+
Pf604	212	10	12	$1.9x\ 10^4$	-
Pf605	138	5	8	$2.1x\ 10^4$	+
Pa606	424	31	34	$0.8x\ 10^4$	+
Pf607	294	19	21	$1.3x\ 10^4$	+
Pf608	350	24	25	$1.0x\ 10^4$	-
Pa609	245	13	14	$1.7x\ 10^4$	-
Pa610	392	27	27	$1.2x\ 10^4$	+
Pf611	196	7	9	$1.8x\ 10^4$	-
Pa612	268	16	17	$1.5x\ 10^4$	+
Pa613	238	12	14	$1.8x\ 10^4$	+
Pa614	260	15	16	$1.6x\ 10^4$	-
Pa615	381	26	28	$0.9x\ 10^4$	-
Pa616	234	12	14	$2.1x\ 10^4$	+
P617	402	28	30	$0.9x\ 10^4$	-
P618	302	20	21	$1.2x\ 10^4$	+
Pa619	420	30	32	$0.8x\ 10^4$	-
Pa620	207	9	11	$1.7x\ 10^4$	-
L.S.D.P = 0.05	7	2	3	------	--------

Table 4: Effects of 4 isolates of *Pseudomonas* on the growth of pigeonpea and on *H.cajani.*, *M.incognita* and *F. udum* under pot condition

Treatments		Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	No. of nodules per root system	Cyst/root system	Galls/ per root system	Wilting index
Control	C	156.7c	106.7d	25.34c	8abc	-	-	-
	H	113.5hi	78.2kl	18.76lm	4cd	102a	-	-
	M	118.9fg	80.8ij	19.17kl	5cd	-	106a	-
	F	107.8j	76.7l	17.62n	4cd	-	-	3
Pf604	C	162.2ab	111.5ab	26.64ab	8abc	-	-	-
	H	122.6e	84.5gh	22.26fg	3d	59d	-	-
	M	128.2d	87.4ef	22.85e	6bcd	-	64d	-
	F	116.2gh	82.3hi	19.44k	6bcd	-	-	2
Pf605	C	164.6a	113.2a	26.85a	6bcd	-	-	-
	H	124.9e	84.4gh	22.44ef	4cd	55d	-	-
	M	130.8d	89.5e	23.33d	5cd	-	59d	-
	F	118.9fg	84.6gh	20.26j	5cd	-	-	2
Pf611	C	160.9b	110.1bc	26.36b	10ab	-	-	-
	H	119.6f	82.3hi	21.11h	8abc	69c	-	-
	M	125.2e	85.6fg	21.97g	7abcd	-	74c	-
	F	113.9hi	80.4ijk	19.16kl	7abcd	-	-	2
Pa616	C	160.1b	108.5cd	25.62c	11a	-	-	-
	H	118.2fg	81.2ij	20.64ij	6bcd	81b	-	-
	M	122.9e	83.7gh	20.85hi	7abcd	-	86b	-
	F	11.4i	78.8jkl	18.63m	7abcd	-	-	3
L.S.D.P=0.05		2.9	2.3	0.44	4	5	7	

C = Control; H = *Heterodera cajani*; M = *Meloidogyne incognita;* F = *Fusarium udum*

*Different letters within one column represent the values that are significantly different at p=0.05

the help of slide and dissolved in sterile water. Number of bacterial cells in the suspension was adjusted as $1.5x10^7$ cells mL^{-1}. Hundred millliter bacterial suspension was poured in 200 g sterilized soil and 100 seeds of pigeonpea were wrapped to provide $1.5X10^7$ bacterial cells per seed.

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 replaced. In control where no bacterial, nematode and fungus inocula were given, water was added in equal volume to the inoculum suspension. The treatments were applied as shown in Table 4 and 5. There were 20 treatments comprising of 5 treatments of *Pseudomonas* isolates in Table 4 (No isolate, Pf604, Pf605, Pf611 and Pa616) each tested with 4 pathogens (Control, *H. cajani* (H), *M. incognita* (M), *F. udum* (F)) and each replicated five times (20x5 = 100 pots). Moreover, there were 25 treatments comprising of 5 treatments of *Pseudomonas* isolates in Table 5 (No isolate, Pf604, Pf605, Pf611 and

Table 5: Effects of 4 isolates of *Pseudomonas* on the growth and wilt disease complex of pigeonpea under pot condition

Treatments		Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)	No. of nodules per root system	Cyst/root system	Galls/per root system	Wilting index
Control	C	156.7c	106.7d	25.34c	8abc	-	-	-
	HM	90.6jk	60.8lmn	14.24mn	2de	92a	96a	-
	HF	79.2n	53.5p	12.55p	2de	84b	-	4
	MF	84.6m	57.2o	13.73o	2de	-	90ab	4
	HMF	65.2o	44.3q	10.42q	0e	78bc	85bc	5
Pf604	C	162.2ab	111.5ab	26.64ab	8abc	-	-	-
	HM	114.6d	78.1e	18.66d	3cde	53gh	58hi	-
	HF	96.8gh	65.6ijk	15.97gh	2de	48hij	-	3
	MF	102.4f	69.7gh	16.77f	2de	-	53ij	3
	HMF	86.3lm	58.7no	14.88kl	2de	45ij	49j	4
Pf605	C	164.6a	113.2a	26.85a	6abcd	-	-	-
	HM	116.3d	79.1e	18.79d	3cde	50hi	52ij	-
	HF	98.5g	67.4i	16.24fg	4cde	47hij	-	3
	MF	105.3f	71.8g	17.24e	4cde	-	54ij	3
	HMF	90.2jk	61.7m	15.25ijk	2de	43j	49j	4
Pf611	C	160.9b	110.1bc	26.36b	10ab	-	-	-
	HM	110.4e	75.1f	17.65e	4cde	65e	70ef	-
	HF	92.7ij	63.2kl	14.94jkl	2de	62ef	-	3
	MF	97.6gh	66.1ij	15.66hi	2de	-	67fg	3
	HMF	80.2n	54.1p	13.98no	0e	57fg	62gh	4
Pa616	C	160.1b	108.5cd	25.62c	11a	-	-	-
	HM	98.1g	66.4ij	15.96gh	3cde	76c	81cd	-
	HF	89.2kl	60.7mn	14.59lm	3cde	72cd	-	4
	MF	94.6hi	64.2jk	15.38ij	0e	-	77de	4
	HMF	77.7n	52.9p	12.62p	0e	66de	71e	5
L.S.D.P=0.05		3.1	2.4	0.46	5	6	7	-

C = Control; H = *Heterodera cajani*; M = *Meloidogyne incognita;* F = *Fusarium udum*

*Different letters within one column represent the values that are significantly different at p=0.05

Pa616) each tested with 5 combination of pathogens (Control, H+M, H+F, M+F, H+M+F) with 5 replicate pots per test combination (25x5 = 125 pots).

Observations: Plants were uprooted 90 days after inoculation of pot experiment and 180 days after inoculation of field experiment. Root systems were gently rinsed and plants were cut with knife 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 shoots and roots separately.. For dry weight determination, shoots were kept at 60°C for 2-3 days before weighing. Number of pods per plant, grain weight per plant, no. of nodules per root system, 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 where, 0 = no disease and 5 = severe wilting.

Statistical analysis: The entire data set was analysed as a single two factor experiment (Pathogens x Bacterial isolates) by the method of Dospekhov[16]. Critical Differences (CD) were calculated at p = 0.05 and Duncan's Multiple Range Test was employed to test for significant differences between treatments.

RESULTS

Twenty isolates of *Pseudomonas* were isolated were isolated from pathogen suppressive soils of pigeonpea fields. These isolates of were characterized on the basis of Gram's reaction, gelatin liquefaction, starch hydrolysis, growth on Kings B medium, growth at 4°C, growth at 41°C, catalase test and levan formation (Table 1). Comparison of these results with the Bergey's manual of Determinative Bacteriology 5 isolates were identified as *P. fluorescens*(Pf604, Pf605, Pf607, Pf608 and Pf611) 13 as *P. aeruginosa* (Pa601, Pa602, Pa603, Pa606, Pa609, Pa610, Pa612, Pa,613, Pa614, Pa615, Pa616, Pa619 and Pa620*)*, 2 isolates P617 and P18 may belong some other species of *Pseudomonas* (Table 1). Carbohydrate fermentation shows that all these isolates were able to ferment glucose with the production of acid and gas while tests of lactose and sucrose show no acid and no gas production.

These 20 isolates were tested for their sensitivity against antibiotics (Table 2) with the aim that isolates having resistance against large number of antibiotics may compete well in soil and may have broader role in disease resistance. Twenty isolates of *Pseudomonas* were found resistant against Ampicillin, Penicillin, Co-trimaxazole and Nalidixic acid except Pa610 and Pa615 which were susceptible to Co-trimaxazole and Nalidixic acid, respectively (Table 2).

Effect of 20 isolates of *Pseudomonas* were observed on the hatching of *M. incognita*, penetration of *M. incognita* and *H. cajani*, antifungal activity and root colonization by these isolates (Table 3). Out of 20, 11 isolates (Pa601, Pa602, Pa603, Pf605, Pa606, Pf607, Pa610, Pa612, Pa613, Pa616, and P618) showed antifungal activity against *F. udum* while nine isolates (Pa601, Pf604, Pf605, Pa609, Pf611, Pa,613, Pa614, Pa616 and P620) caused greater colonization of pigeonpea roots. Moreover, 6 isolates (Pf604, Pf605, Pa609, Pf611, Pa616 and P620) have adverse effect on hatching and penetration of both nematodes. Finally, 4 isolates of *Pseudomonas* namely Pf604, Pf605, Pf611 and Pa616 were selected as potential biocontrol agents after greenhouse assay on the basis of antifungal activity, greater root colonization by these isolates and greater adverse effect on hatching and penetration of *M. incognita* and *H. cajani* (Table 3)

Four isolates (Pf604, Pf605, Pf611 and Pa616) found potential in green house assay test were evaluated under pot condition for the biocontrol of wilt disease complex of pigeonpea. Inoculation of isolates of *Pseudomonas* increased plant growth of both pathogen inoculated and uninoculated plants but increase in plant growth caused by inoculation by *Pseudomonas* isolates was greater in pathogen inoculated plants than in plants without pathogen (Table 4). Out of 4 isolates of Pseudomonas used isolate Pf605 was best in increasing plant growth followed by Pf604, Pf611 and Pa616. Inoculation of pathogen had an adverse effect on nodulation but inoculation of Pseudomonas isolates had no effect on nodulation. Isolate Pf605 and Pf604 had almost similar adverse effect on cyst formation and root galling followed by Pf611 and Pa616. Isolated Pf605, Pf604 and Pf611 reduced wilting index while Pa616 had no effect on wilting index (Table 4).

When these 4 *Pseudomonas* isolates were used against plants simultaneously inoculated with 2 or more pathogens also increased plant growth (Table 5). Isolate Pf605 caused greater increase in plant growth followed by Pf604, Pf611 and Pa616. Simultaneous inoculation of pathogens caused an adverse effect on nodulation but inoculation of *Pseudomonas* isolates had no effect on nodulation. Isolate Pf605 caused greater reduction in cyst formation and root galling followed by Pf604, Pf611 and Pa616. Isolates Pf605, Pf604 and Pf611 reduced wilting index implants simultaneously inoculated with pathogens while isolate Pa616 had no effect on wilting index (Table 5).

DISCUSSION

Four isolates of *Pseudomonas* were selected after laboratory and screen house tests for the biocontrol of wilt disease complex of pigeonpea under pot condition. Out of 20 isolates, 11 isolates have shown antifungal activity while 18 isolates have shown resistance against 4 antibiotics out of 6 tested. Isolates having resistance against higher number of antibiotics may successfully compete and survive in soil and may have a broader role in disease control. In addition, these 9 isolates (Pa601, Pf604, Pf605, Pa609, Pf611, Pa613, Pa614, Pa616 and P620) also caused greater root colonization and 6 isolates (Pf604, Pf605, Pa609, Pf611, Pa616 and P620) had greater inhibitory effect on hatching and penetration of nematodes. Beside this, isolates Pf604, Pf605, Pf611 and Pa616 produced greater fluorescence than other *Pseudomonas* isolates in Kings B medium. Four isolates namely Pf604, Pf605, Pf611 and Pa616 which have greater root colonization, resistance against 4 common antibiotics, greater inhibitory effect on hatching and penetration of nematodes, greater fluorescence produced in Kings B medium and inhibitory effect on *F. udum* were selected as potential isolates after green house assay.

Isolate Pf605 provided better biocontrol than other isolates used. Fluorescent pseudomonads are known to suppress Fusarium wilt anf othe pathogens of various plant species[17]. For many pseudomonads, production of metabolites such as antibiotics, siderophores and Hydrogen Cyanide (HCN) is the primary mechanism of biocontrol[18]. *Pseudomonas* produce a polar substance heat labile, sensitive to extreme pH values causing in vitro juvenile mortality of *M. javanica*[19]. Moreover, many strains of pseudomonads can indirectly protect the plants by inducing systemic resistance against various pests and diseases[20-22]. Fluorescent pseudomonads play a critical role in naturally occurring soil that is suppressive to Fusarium wilt[23] and may be used successfully for the biocontrol of many plant pathogens[5,24,25].

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