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Isolation, identification and screening of PGPRs and evaluation of their effectiveness on pigeon pea growth with regard to disease complex management

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Abstract

Plant disease is major problem to global food production. Employments of excess of chemical pesticides in the management of plant disease became major problem to main table agronomics. PGPRs is usually known for plant protection as well as for enhancing crop productivity. The implementation of PGPR can reduce the requirement of pesticides and chemical fertilizers which is very important for sustainable agriculture. This is proved by the researchers PGPRs enhancing the crop yield via direct and indirect mechanism of plants. Pseudomonas fluorescnes is an important PGPR which have great importance in systemic resistance ,plant growth promotion, and biotic management of plant disease and plant growth promotion. Farmers applied large amount of chemical pesticides and food production. PGPR are most important agents in the reduction of disease and increment of growth and yield of plants. Seven strains of Pseudomonas fluorescnes were separated from the soil of pigeon pea field. Pseudomonas fluorescens have significant importance in the increment of plant health and growth. The current study aim to isolated and identify the Pseudomonas fluorescens from the soil their role in the control of wilt disease of pigeon pea. PGPR suppresses plant disease by production of IAA, sulphur, phosphorus, and siderophores. Pseudomonas fluorescens significantly control the plant pathogens. Pseudomonas fluorescens produced the solubilized phosphorus, potassium and fix the atmospheric nitrogen. Pseudomonas fluorescens not only protected plant from pathogens but also enhanced the soil fertility. PGPRs are the important microorganism produced the molecules which enhanced production plant hormone, growth and development.

Keywords: PGPRs, Pseudomonas fluorescens, Siderophores, IAA, Meloidogyne incognita, Heterodera cajani.

Introduction

Pigeon pea [(*Cajanus cajan*) (L.) Millspp.] regularly is known as tur, dal, or red gram and have second position among the pulse crop in India which is essential food legume crop in semi-arid regions worldwide[1]. Due to presence of high protein content it is highly recommended component of our diet. *Fusarium udum*, *Meloidogyne incognita* and *Heterodera cajani* are the detrimental parasite of pigeon pea[2] and responsible for the wilt disease complex of pigeon pea[3,4].

PGPR (Plant growth promoting rhizobacteria) are more suitable agents in agriculture industry and have lots of benefits such as environmental cleanup strategies, heavy metal detoxification, degradation of pesticides phosphate solubilization, defense against pathogens, salinity tolerance, secretion of plant growth hormones, and nitrogen fixation[5]. Various kinds of researchers has been reported that PGPRs are most important agents in the mass production of crop. PGPRs also known as biostimulants which are effectively used to increase the plant growth and yield and food security[6]. PGPRs have great importance in survival and adapt under challenging environmental state and became extra flexible to stress, they are very helpful in regulation of water content in the soil, nutrient acquisition, regulating osmotic balance and ionic homeostasis[7]. PGPRs exhibits synergistic or antagonistic interaction with rhizosphere microorganism and soil that indirectly increase the plant growth[8].

PGPRs are suppressed the plant pathogens growth and increased crop production and area surrounding the root surface where microbial activity is highest is known as rhizopsheric area[9]. PGPR when applied on the crop and seed it enhanced the yield and growth of plant[10] and which free the living rhizobacteria that strongly remain attached to rhizospheric plant roots. Due to release of metabolite, PGPR generate plant growth promoting effect in plant then enhanced the growth. PGPR representing the microbial group colonizing the roots, and which have capacity to directly indirectly enhance the plant growth and protected from damage or disease and pathogen attack. PGPR have great role in different mechanism such as immobilization of mineral nutrient synthesis of organic matter, mineralization of organic matter, synthesis of organic matter that increased the fertility of soil[11]. PPGR as biocontrol used several mechanism like production of hydrolytic enzyme and inhibitory compound against the phytopathogens.

Various strains of *Bacillus, Stenophomonas*, and P*seudomonas* have been utilized to increase the plant growth and in the management of plant disease [12,13]. Lots of rhizobacteria are utilized as biocontrol agents[14], but *Pseudomonas* spp. more suitable than the other biocontrol agent[15]. Different strains of *Pseudomonas* produced metabolites like, HCN (hydrogen cyanide), antibiotics, and siderophores which are primary mechanism of bio control agent[16]. Different PGPRs like *Bacillus*, *Rhizobium*, *Xanthomonas* and *Pseudomonas* reported and evaluated against the *F. udum*. *Fluorscent pseudomonads* implemented as seed piece or seed inoculant reduced the growth of soil borne and enhanced yield of crop[17].

Pseudomonas fluorescens is a bacterium known for its remarkable capacity to stimulate plant growth and suppress a wide range of soil-borne pathogens. Its multifaceted role in soil health has made it an invaluable asset in sustainable agriculture, particularly in the management of soil-borne diseases. With the increasing concerns over chemical pesticide usage and their detrimental environmental impacts, there is a growing demand for eco-friendly alternatives. Among the promising solutions, *P. fluorescens* stands out due to its efficacy in suppressing pathogens and improving plant resilience through a variety of mechanisms[18]. *P. fluorescence* strain exhibit biocontrol activity against the suppression of plant disease by protecting root and seed from the plant pathogens and enhanced the plant growth[19, 20]. *P. fluorescense* have the capability to produce IAA, HCN, solubilize the phosphate, siderophores, therefore act as biocontrol agent against the bacterial disease of potato and tomato and increased the plant growth[21,22]. The objective of this study was isolation, identification and screening of potential PGPRs, and evaluation of their effectiveness on wilt disease complex of pigeon pea.

Materials and Methods

Collection of soil sample: Soil samples were collected from the rhizosphere of pigeon pea at different site Atrauli, Iglas and Sasni of Aligarh, U.P., India.

Colony morphology, biochemical test and pigment production: King's B agar media was used for the identification of colony morphology and pigment production after 24 to 48 hours at 28±2^{o}C [23]. Biochemical test was done by Gram stain and identified according to Bergeys manual of determinative of Bacteriology[24]. Culture of Bacteria store at 4^{o}C and maintained on the respective slants.

Gram staining procedure: Chemical agents: i. 0.1% basic fushchin, ii. Acetone/ethanol (50:50 V:V), iii. Iodine solution, iv. Crystal violet.

Procedure: i. A drop of suspended culture was used as an inoculation loop full transfer to a microscope slide. ii. The colony containing Petri dish had some loophole A Petri dish containing the colony had some loopholes of water adjoin tomake easy to the transfer of a small quantity of colony to the slide for examination. iii. Just a minimal quantity of culture was required. On the inoculation of loop of culture was visible and indicated an excessive collection of culture. iv. With the help of inoculation loop culture was spread into an even, thin film over a 15mm. When examining more than one culture slide have four minimal streaks. Slide was air-dried using heat over a low flame. To prevent forming ring pattern or overheating the slide was moved in a circular motion over the flame. The heat helped the minimizing culture loss during rinsing and cells adhere to the glass slide.

Gram Staining: i. The excess stain was rinsed with water and after 10-20 seconds, the stain was poured off. The object without losing the fixed culture to wash off the stain. ii. Solution of iodine was used for 10-20 seconds to cover the streaks for dye fixing. The solution of iodine solution poured off on the slide and then washed tap water. Extra water was removed outside. iii. For the decolorization, mixture of acetone and ethanol were adjoining to the slide then after 5 seconds the slide was washed with tap water. After the addition of the decolorizer the solvent was flowed over the slide which was the clarification of the reduction of decolorization from gram positive cells. iv. Basic fuchsin solution was used the counterstained of smear for 40-50 seconds then fuchsin solution was then rinsed with water and extra water was blotted with blotting sheet. In the end removal of extra water then slide was air-dried.

Slide analysis under the microscope: i. By using oil immersion the slide was examined under a microscope. ii. At first the slide was observed under the X40 objective to investigate to smear followed by X100 oil immersion. iii. Each zone of slide required thorough examination, as certain regions could yield variable or incorrect results. During the solvent treatment bacterial cells retains the crystal violet which is main aim of gram staining. Gram negative bacteria contain higher lipid content and gram positive bacteria higher peptidoglycan. Bacteria dyed with crystal violet dissolved their primarily lipid layer from gram negative, and the nlayer gram negative loses the stain due the dissolution of lipid layer. Due to dehydration of solvent violet-iodine formed the complex gram-positive bacteria close their cell pores and bacteria remain stained. For the gram staining fuchsin stain gives decolorization gram negative bacteria pink colour which is easy in identification[25].

Antibiotic sensitivity test: According to Baur et al.,[23] and Antoun et al.,[24]disc diffusion method was used for determination of sensitivity against antibiotic of *P. fluorescens*.

Phosphate Solubilization: Agar containing precipitation tricalcium phosphate was used for the phosphate solubilizing test was conducted qualitatively by plating the bacteria. Incubation of Pikovskaya agar with insoluble tricalcium phosphate was done at 28^{o}C for 120h and then twirl of fresh bacterial culture was spread on the middle of agar plate which was modified with this media. Positive phosphate solubilization

is represented by the presence of halo zone around bacterial colonies.

Fabrication of HCN (Hydrogen Cyanide Production) and IAA: Fabrication of HCN was analyzed by the procedure of Lorck[25]. For the isiolation of bacteria isolated adding the adding with glycine 4.4g/l in on nutrient agar media with synchronized addition of in 0.5% picric 1% Na_2CO_3 in upper lid of petriplate filter paper was soaked and sealed with interchange in the color was characterized at 32±2°C. Changing in the color from yellow to light brown (++) or strong (+++) brown exhibited the production of HCN. For the identification of IAA King's B medium amended with nitrocellulose membrane disk (82nm) saturated with Salkowask's reagent[25].

ACC (1-aminocyclopropane-1-carboxylate) deaminase: Activity of ACC- deaminase was analyzed though ACC hydrolyze deaminase into α-ketoglutarate was stained using the 2,4-dinitrophenylhydrazine reagent to determine the content of α-ketoglutarate in the bacterial solution[26]. Coomasie Blue (Breadford) assay was used for detection of standard protein content in Bovine serum albumin. Every assay was replicate three lines. The activity of ACC-deaminase was demonstrated as the quantity of α-ketoglutarate fabricated per mg of protein per minute.

Siderophore production: In the King's B medium Chromoazurol S act as an indicator dye, solution of Fe^{3+}, and hexadecyl trimethyleaniume bromide was used for analysis of Siderophore production. Siderophore production is appeared as the orange halo zones around which is clear indication of siderophore production[27]. The agar containing Chrome Azurol S (CAS) dys formed complex with Fe^{3+} turn into blue colony.

Rising of test plants: Pigeon pea seeds were used in this experiment. 0.1% mercuric chloride used for the sterilization of seeds for 2 minute rinsed three times with double distilled water. Soil in 15cm earthen pots soil sterilized pots and 5 seeds were sown in each pot.5 seeds were sown in each pot and thinning was done after one week germination to maintain single plant and plants were kept in the green house at 30-40^oC temperature in a factorial block design and uninoculated plant serve as control. Water poured in pots as much needed. After inoculation and treatment the experiment was terminated 90 and each treatment repeated 5 times.

Fungal inoculums: Potato dextrose agar (PDA) media was used for the maintenance of *F. udum* culture was separated from the infected pigeon pea root. Richards's liquid medium used for the reculture of *F. udum* maintained at 25^oC for 15 days[28] for the inoculation. Mycelium was collected on blotting sheet nutrient was removed by pressing it between two fold of blotting sheets and also removed extra water. 1L distilled water was used for maceration of 100g mycelium and 10ml of solution having 1g fungus inoculated around roots. The population level of *F. udum* in the form of CFU was calculated in such way fungus contained 2.0×10^4 viable cell.

Nematode inoculums: Pure culture of *M. incognita* was maintained the from *Solanum melongena* roots which was heavily infected and huge amount of egg-masses and sterilized forceps are used for the picking of egg masses. The egg masses placed in Petri dishes with 15 coarse sieves 10m in diameter water just deep enough to come in contact with egg-masses carried crossed layers of tissue paper and with the help of distilled water egg masses washed. Fresh water added to petri plate and every 24hhatched juveniles were collected from the Petridishes. Concentration of *M. incognita* modified in water suspension and each ml this suspension (i.e., 1000 freshly hatched juveniles) was poured around the seedling.

Soil and root collected from pigeon pea field for the preparation of inoculum of *H. cajan*. 100 mesh sieves for isolation of cyst soil and root were examined for the cyst and root exudates of pigeon pea placed for hatching. Inoculation of juveniles done by at the rate of 500 juveniles per plant[29].

Bacterial inoculums: Nutrient agar media is used for the culture of isolates of *P. fluorescens* (IS-1, IS-2, IS-3, IS-4, and IS-5). With the help of slide each isolates was and then dissolved in distilled water. Each isolate was inoculated into each and every pot on every side of the pigeon pea seedling and 10mL of this suspension 1mL contains about $1.5X10^7CFU/mL$[30].

Procedure of inoculation: One week old healthy pigeon pea seedling was used for the inoculating the bacteria. Inoculation of bacterium, nematodes and fungus was done by following mechanism without damaging the root system soil everywhere the root surface was detached carefully. The solutions of inoculum were poured all over the root and then covered with the soil.

Experiment Design: The experiment was conduct in a completely block design uninoculated served as control (without pathogens) and inoculated with pathogens. There were 5 treatments of *Pseudomonads* isolates i.e., IS-1, IS-2, IS-3, IS-4, and IS-5 each tested on pathogen *M. incognita*, *H. cajani* and *F. udum* and each treatment replicated 5 times.

Estimation of pathogen (nematode and fungal) parameters: Technique of Halbrook et al., was used in calculating of egg masses[31]. Root galls and number of cyst were counted visually. Root knot or root galling determined through 0-4, where 0 = no infection, 4 = very severe infection (75-100%), 3 = sever infection (51-75%), 2 = moderate infection (26% - 50%), 1= slight infection (1-25%)[32]. Wilting index was measured by calculating the disease severity on 0-5 scale where 5 = severe wilting, 0 = no disease.

Estimation of plant growth, physiological and biochemical parameters: Physiological and morphological parameters of tested plant like, plant dry weight, plant fresh, number of pods, shoot length; root length number of nodules was recorded after

90 days. Plant dry weight, plant fresh weight was measured through the weighing balance in g, root length; shoot length measured by the scale in cm and number of pods counted visually. Plant growth parameters have been recorded after the crop termination.

Determination of chlorophyll: 1gram finely leaves were taken cut fresh leaves and grinned with 20-40ml of 80% acetone and then centrifuge at 5000 – 10000 for 5 minutes. Method was replicated till residue became colorless and the supernatant was transferred. The absorbance of solution was recorded against the solvent (acetone blank) at 645nm and 663nm. Total chlorophyll content were calculated by using following equation[33]-

Total chlorophyll= 20.2 (A645) + 8.02 (A663) ×V/1000×W

Where, W=fresh weight of tissue extracted, A=absorbance at specific wavelength, and V=final volume of chlorophyll and carotenoids extract 80% acetone

Estimation of Proline: Proline content in pigeon pea was analyzed by procedure of Bates et al.[34] in pigeon pea. Total proline content was estimated on the basis of development of a brick red coloured proline-ninhydrine complex in acidic medium. By using a mortar pestle plant sample was homogenized in 5ml of sulphonic acid (3%), then homogenite sample was filtered and then filtrate was employed for the determination of proline amount. Plant sample was taken in test tube which was 2ml then glacial acetic acid (2ml) and ninhydrin (2ml) reagent were dissolved in plant sample and for 30 minutes heated at 100°C. Toluene (6ml) mixed and removed to a separate and its absorbance at 520nm in a spectrophotometer against toluene blank.

Estimation of phenolic: Folin-Ciocalteu method was employed for analysis of total phenolic content. Folin-Ciocalteu (0.5mL) reagent and 3mL distilled water mixed thoroughly 3 minute and 200µL of crude extract and then add sodium carbonate2mL of 20% (w/v). This solution was leaved for a further 60 minute. The outcome were represented as mg of gallic acid equivalent per g dry weight and the overall phenolic amount was measured by the calibration curve[35].

Statistical Evaluation: All data was recorded as mean ± standard error of five replicates determined, and evaluated as employing ANOVA with significant differences between means determines at $P \leq 0.05$ and measured with Duncan's multiple range test using the statistical package for 14(SPSS).

Results and Discussion

Soil sample was collected from the rhizosheric soil of pigeon pea. PGPRs isolated were identified based on their biochemical and morphological characteristic as given in Bergey's manual of systemic bacteriology. Isolated 10 samples belonging to genus *Pseudomonas* spp. were recognized based on these characters gram negative, clear colony, and rod shape, irregular or regular borders. Out of 10, only 5 samples showed yellowish and greenish in colour under the UV light string test with 3% KOH. Isolates of *P. fluorescens* produced IAA (+++) which is strong indication of production IAA (Table-1). Additionally, isolates of *P. fuorescens* showed highest activity to produced HCN (Table-1). Similarly, *P. fluorescens* strain positively (+) produced ACC deaminase. Moreover, all strains of the *P. fluorescens* exhibited PGP activity, though at varying concentrations.

Table-1: Morphological and Biochemical Characterization of *Pseudomonas flourescens* isolates.

Characters	*Pseudomonas fluorescens*
Gram reaction	-
Pigmentation	Greenish, Yellowish and Whitish
Siderophores Production	+
Phosphate solubilization	+
King'B Medium	+
HCN	+++
IAA	+++
ACC deaminase	+

Cultural and morphological characterization of PGPRs: Isolated 5 isolates of *Pseudomanas* sp. were separated from the repressive soil of pigeon pea fields. These isolates were distinguished and differentiated based on the basis of Gram's reaction and growth on King's B media. Isolates of *Pseudomonas sp.* exhibited smooth and small colonies white and yellow pigments. Bergey's manual of 'Determinative Bacteriology' was used for the comparison of these results. Different isolates were tested for sensitivity against the isolates. 7 isolates of *P. fluorescens* (IS-1, IS-2, IS-3, IS-4, and IS-5) showed resistance against the number of antibiotics except Pf610 Table-2.

Siderophores production: Isolates of PGPR (*P seudomonas* sp.) was tested and found showing production of siderophores in CAS agar medium which is blue in colour (Figure-3). PGPRs colony turned into orange halo zones which is clear indication of siderophore production. Production of siderophore is the formation of orange halo zone against the dark blue which is the clear indication. Orange halo zone measured through the diameter. *Pseudomonas* isolates exhibited > 4mm diameter halozones and produced more siderophores (Figure-3).

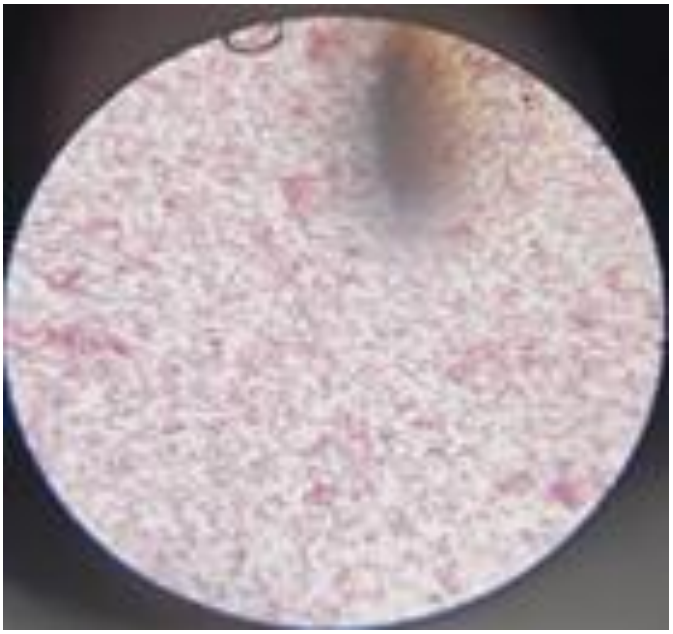

Figure-1: Gram staining of PGPR.

Figure-2: Isolation of isolates of *Pseudomonas sp.* on King's B medium.

Table-2: Antibiotic test for isolates of *Pseudomonas fuorescense.*

Isolates	Nalidixic Acid Na^{30}	Co-trimozoleCo^{25}	PenicillinP^{10}	AmpicllinA^{10}
IS-1	Resistance	Resistance	Resistance	Resistance
IS-2	Resistance	Resistance	Resistance	Resistance
IS-3	Resistance	Resistance	Resistance	Resistance
IS-4	Resistance	Resistance	Resistance	Resistance
IS-5	Resistance	10nm (Susceptible)	Resistance	Resistance

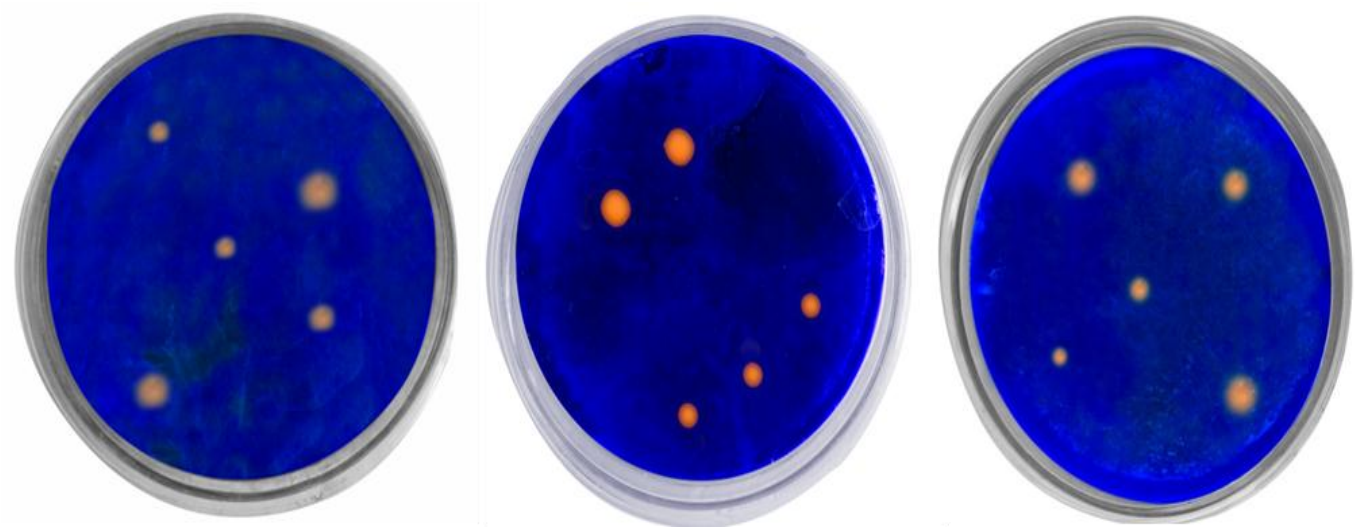

Figure-3: Production of Siderophores by isolates of *Pesudomonas* sp.

Figure-4: Phosphate solubilization by isolates of *Pseudomonas sp.*

Phosphate solubilization: For the plant growth and development phosphorus is the vital element. The selected *Pseudomonas fluorescens* strains were determined as better phosphate solubilizers based on the screening of their screening activity by their clear zone around colonies on pikovskayes agar plate (Figure-4).

Effect on Plant growth parameters: PGPRs showed the beneficial impact on plant growth related parameters. Application of different isolates of *Pseudomonas fluorescence* T3(IS-1), T4(IS-2), T5(IS-3), T6(IS-4), and T7(IS-5) increased the plant fresh weight, plant dry weight, number of pods, shoot length, root length number of nodule, total chlorophyll, total carotenoid, total proline, and total phenolic. In detail, IS-1 (T3) enhanced the plant fresh weight 53.12%, plant dry weight 61.23%, number of pods 4.98%, total chlorophyll 65.10%, total carotenoids 65.33%, proline 29.52% and phenolic 17.23% as compared to inoculated control (Table-3 and 4). Likewise, IS-2 (T5) enhanced plant fresh weight 50.11%, plant dry weight 61.85%, number of pods 60.78%, shoot length 53.55%, root length 37.96%, number of nodule 37.78%, total chlorophyll 73.36%, total carotenoid 79.12%, total proline 43.20%, and total phenolic 2.85% as compared to inoculated control (Table-3 and 5). IS-4 (T6) inoculation enhanced the plant fresh weight 64.78%, plant dry weight 63.10%, number of pods 61.40%, shoot length 68.11%, root length 47.63%, number of nodule 61.47%, total chlorophyll 79.55%, total carotenoid 85.22%, total proline 65.44%, and total phenolic 21.11% as compared to inoculated control (Table 3 and 4). IS-5 (T7) enhanced the plant fresh weight 47.78%, plant dry weight 63.89%, number of pods 61%, shoot length 61.55%, root length 19.36%, number of nodule 54.05%, total chlorophyll 69.63%, total carotenoid 57.12%, total proline 25.45%, and total phenolic 3.96% as compared to inoculated to control. The treatment (T4) IS-2 significantly increased the Plant fresh weight 27.52%, plant dry weight 66.12%, number of pod 64.85%, shoot length 70.21%, root length 60.11%, number of nodule 72.30%, total chlorophyll 84.85%, total carotenoids 85.78%, total proline 45.32%, and total phenolic 80.33% as compared to inoculated control (Table-3 and 4).

Pathogen related parameters: Data given in Table-4 revealed that different isolates of *Pseudomonas fluorescens* at various treatments (T3) IS-1, (T4) IS-2, (T5) IS-3, (T6) IS-4, and (T7) IS-5 reduced the pathogen related parameter. IS-1 (T3) reduced no. of egg mass 27.11%, no. of galls/root system 12.32%, no. of cyst/root system 52.10%, total nematode population at J2/kg soil 66.23%, cyst/kg population 53.96%, root knot index 73.41%, wilting index 50.21% and disease severity 34% as compared to inoculated control (Table-5). IS-3 (T5) reduced number of egg mass 55.85%, number of galls/root system 54.23%, number of cyst/root system 40.21%, total nematode population at J2/kg soil 46.50%, cyst/kg population 50.42%, root knot index 35.47%, wilting index 70.69% and disease severity 52.23% as compared to inoculated control (Table-5). IS-4 (T6) reduced no. of egg mass 70.30%, no. of galls/root system 54.45%, no. of cyst/root system 82.21%, total nematode population at J2/kg soil 67.69%, cyst/kg population 59.21%, root knot index 75.11%, wilting index 70.32% and disease severity 58.11% as compared to inoculated control as compared to inoculated control (Table-5). IS-5 (T7) reduced no. of egg mass 64.03%, no. of galls/root system 29.32%, and no. of cyst/root system 74.11%, total nematode population at J2/kg soil %, cyst/kg population 33.31%, root knot index 25.74%, wilting index 62.11% and disease severity 38% as compared to inoculated control Table 4.Overall, IS-2 (T4) exclusively reduced the no. of egg mass 58.32%, no. of galls/root system 56.32%, no. of cyst/root system 86.11%, and total nematode population at J2/kg soil 75.59%, cyst/kg population 72.45%, root knot index 91.23% and wilting index 75.11% as compared to inoculated control (Table-5).

Discussion: Different biocontrol agents are successfully utilized in the management of phytopathogens and also used as an alternative to chemical fungicides, rhizospheric microbiota possessing rhizospheric competence in the nonexistence of targeted crop disease management and effective root colonization[36]. Growth promoting rhizobacteria have great importance in stimulation of plant productivity, growth, increasing root biomass, nutrition through improvement of nutrient acquisition and in natural ecosystem[37]. Actual process of PGPR in stimulation of plant growth is still unknown but different mechanism of action like repression of harmful organism, phosphate solubilization promotion of mineral nutrient uptake and production of phytohormones are participated in plant growth promotion[38,39]. Siderophores is the most important mechanism in biocontrol which is produced by the PGPR including *Pseudomonas fluorescens* and siderophores showed more affinity with iron. The growth of plant pathogen suppressed because of iron in the environment[40].

Table-3: Effect of *Pseudomonas* isolateson plant growth and biochemical parameters of pigeon pea infected with *F.udum, M. incognita* and *H. cajani.*

Treatments	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Shoot length (cm)	Number of Pods	Number of Nodule	Total Chlorophyll (mg/g)	Total Carotenoid (mg/g)	Proline (µg/g)	Phenolics (µg/mL)
T1	88.12^{a}±10.18	24.30^{a}±9.10	25.66^{a}±0.38	82.74^{a}±12.36	25.44^{a}±3.78	84.63^{a}±15.74	2.86^{a}±0.073	1.99^{a}±0.003	0.183^{b}±0.000	1.40^{d}±0.005
T2	24.23^{c}±5.96	7.45^{b}±3.96	10.45^{c}±0.33	22.11^{e}±8.32	8.53^{c}±2.98	23.25^{d}±9.56	0.58^{d}±0.123	0.25^{e}±0.047	0.103^{a}±0.025	3.10^{ab}±0.033
T3	55.14^{b}±9.25	18.78^{a}±8.45	15.46^{bc}±1.17	40.23^{d}±11.79	19.80^{b}±8.27	41.33^{cd}±13.14	1.22^{c}±0.049	0.98^{cd}±0.005	0.147^{b}±0.011	3.74^{cd}±0.222
T4	83.78^{a}±11.52	23.96^{a}±2.48	25.77^{a}±0.33	80.25^{a}±15.66	24.12^{a}±9.29	84.50^{a}±15.20	2.99^{a}±0.003	1.95^{a}±0.024	0.183^{b}±0.001	3.91a±0.037
T5	48.30^{c}±7.85	18.43^{a}±6.10	16.48^{bc}±2.14	47.55^{d}±8.89	20.33^{b}±6.45	60.33^{bc}±9.36	2.17^{b}±0.113	1.21^{bc}±0.200	0.152^{b}±0.005	3.19^{bc}±0.318
T6	68.31^{ab}±9.78	19.74^{a}±5.69	19.39^{ab}±1.45	69.36^{b}±13.56	21.32^{ab}±5.36	71.47^{ab}±7.45	2.87^{a}±0.031	1.72^{ab}±0.152	0.170^{b}±0.005	3.78^{bc}±0.115
T7	46.09^{bc}±10.89	19.30^{a}±6.85	11.38^{bc}±2.54	57.30^{c}±10.10	21.30^{ab}±5.58	54.23^{bc}±5.36	1.91^{b}±0.018	0.59^{de}±0.056	0.129^{b}±0.004	3.22^{cd}±0.313
LSD(P≤0.05)	3.1	2.6	1.6	3.9	1.3	1.6	0.4	0.9	1.4	1.7

T1= Control; T2= *F udum + M incognita + H cajani;*T3= IS-1; T4= IS-2; T5= IS-3; T6= IS-4; T7=IS-5

Table-4: Effect of *Pseudomonas* isolates on pathogen-related parameters of pigeon pea infected with *F.udum, M. incognita* and *H. cajani*

Treatments	Egg Masses	Galls/root system	Cyst/root system	Total nematode population		Root knot index	Wilting Index	Disease severity percentage
				J2 population/kg soil	Cyst population/kg soil			
T1	0^{e}±0	0^{e}±0	0^{e}±0	0^{c}±0	0^{c}±0	0^{e}±0	0^{c}±0	0^{d}±0
T2	87.09^{a}±0.83	192.12^{a}±1.53	323.12^{a}±8.38	6521.02^{a}±46.23	674.7^{a}±16.02	4^{a}±0.19	5^{a}±0.19	97.12^{a}±0.33
T3	63.25^{b}±0.96	168.78^{b}±3.46	155.56^{b}±5.58	2167.12^{bc}±24.00	316.12^{b}±44.09	2.3^{b}±0.69	2.5^{ab}±0.50	64.10^{b}±4.03
T4	36.96^{d}±2.54	84.69^{d}±1.85	45.78^{d}±10.40	1600.56^{bc}±59.23	183.30^{bc}±30.12	0.33^{de}±0.19	1^{bc}±0.33	31.33^{c}±3.42
T5	56.69^{bc}±0.57	93.10^{d}±1	64.45^{d}±9.52	3503.96^{b}±23.17	333.45^{b}±42.33	2.6^{babc}±0.50	1.5^{b}±0.50	46.39^{bc}±8.38
T6	51.12^{c}±4.19	87.36^{d}±1.67	58.23^{d}±8.38	2233.23^{bc}±48.07	276.85^{b}±46.89	1^{cde}±0.47	1.5^{bc}±0.57	60.00^{b}±3.33
T7	31.11^{d}±2.18	136.78^{c}±2.58	83.90^{c}±1.53	1933.74^{bc}±25.14	240.32^{bc}±29.06	3^{abab}±0	2.5^{ab}±0.33	42.23^{bc}±3.86
LSD(P≤0.05)	1.8	2.7	2.9	2.9	2.0	0.9	0.5	1.3

T1= Control; T2 = *F udum +M incognita + H cajani;* T3 = IS-1; T4 = IS-2; T5 = IS-3; T6 = IS-4; T7 = IS-5.

Different PGPR are utilized in the management of plant disease, however, *Pseudomonas fluorescens* commonly used as biofertilzer and potential biocontrol agent in agriculture[41]. In comparison to non rhizospheric soil rhizospheric contained phosphate solubilizing bacteria. Bacteria belong to genus *Pseudomonas* spp. significantly known as phosphate solubilizing bacteria[42]. IAA has great importance in development and growth of plant and functioned growth hormone. IAA production varied affected by and growth stage, culture condition, and substrate availability[43]. Due to different plant growth regulator and production of siderophores, PGPR improved the plant growth parameters[44,45]. Seed treated with *Pseudomonas fluorescence* reduced the root rot and nematode multiplication and increased pod yield[46]. According to Siddiqui et al.[47] reported that *Pseudomonas fluorescens* enhanced nodulation, phosphorus, plant growth and reduced wilting in plants and not enhanced the nodulation but reduced the nematode multiplication.

PGPRs isolated from the soil rhizosphere soil samples of pigeon pea, were used *F. udum*, *H. cajani* and *M. incognita* which is responsible for the wilt disease complex of pigeon pea. Biocontrol agents are efficient rhizospheric microorganism in inhibition of foliar and root disease of different plants[48,49]. Lots of rhizobacteria are utilized in management of plant disease but among them *Pseudomonas* spp. is the best biocontrol agent. Environmental factor have great role in improvement level and reliability and level biocontrol and biosynthesis of antimicrobial compound[50]. Different researchers reported that *Pseudomonas* increased of resistance mechanism, increased the root colonization, plant growth, and inhibited the pathogen growth by competing of Fe (III), production of diffusible or volatile product which have great role in the suppression of pathogen[47,51,52]. According to Siddiqui and Shakeel[53] Pf604, Pf605, and Pf611 exhibited negative impact on wilting disease complex of pigeon pea pathogens such as these strains reduced galling, cyst, and wilting which is caused by the *M. incognita*, *H. cajani*, and *F. udum* respectively. My results are in concurrent with the aforementioned results. Additionally, Pf736 and Pa737 reduced the pathogens growth by the production of siderophore and HCN[54]. These variations in the ability to produce siderophores is consistent with earlier reports[55].

Different strains of *Pseudomonas* spp. have capability indirectly protect the plant from pathogen attack by increasing the systemic resistance mechanism against the different disease and pest[56]. These rhizobacteria have shown potential results in suppression of plant diseases caused by these pathogens, and also enhanced the plant growth activity[57], naturally found in soil and can be successfully utilized as biocontrol agent against the plant pathogens[58,59].*P. fluorescens* produced lipopeptide like, visconsinamide and pseudofactin II tensin antimicrobial compound which enhanced the plant immune system against the plant pathogens[60]. *P. fluorescens* used as plant growth promoter in supportable reduction of plant disease over the chemical pesticides[61].

Conclusion

This study is revealed that potential of *Pseudomonas fluorescens* isolates as plant growth-promoting rhizobacteria (PGPR) to enhance pigeon pea (*Cajanus cajan*) growth and control soil-borne pathogens, including *Fusarium udum*, *Meloidogyne incognita*, and *Heterodera cajani*. The research involved morphological and biochemical characterization of the isolates, uncovering beneficial traits like siderophore production and phosphate solubilization, which are crucial for nutrient availability. However, antibiotic resistance profiles revealed that all isolates showed resistance to commonly used antibiotics, raising concerns about their environmental implications.

The results showed that *Pseudomonas fluorescens* significantly enhanced plant growth parameters, such as fresh and dry weights, root lengths, and chlorophyll content, in comparison to the control groups. Notably, the isolate IS-4 showed the most pronounced effects on growth metrics. Additionally, the isolates effectively reduced the population of pathogens and related disease indicators, with IS-4 achieving the highest reduction in egg masses and galls. These findings support the potential of *Pseudomonas fluorescens* as a sustainable biocontrol agent and highlight its importance in promoting plant strength against diseases.

Outcome, this study contributes to the understanding of how *Pseudomonas fluorescens* can be utilized in sustainable agricultural practices, providing a foundation for future research aimed at applying these biocontrol agents in field conditions to mitigate the impacts of soil-borne diseases.

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