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APPLICATION OF ENDOPHYTIC FLUORESCENT *PSEUDOMONAS* FOR MITIGATING THE PROLIFERATION OF ROOT-ROTTING FUNGI OF *CICER ARIETINUM* L.

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ASBTRACT

The focus of the current study is on the antagonistic capability of fluorescent *Pseudomonas* in both *in-vitro* and *in-vivo* by observing its inoculation impact in enhancing growth of chickpea (*Cicer arietinum*) inoculation. There were twenty five isolates of endophytic fluorescent *pseudomonas* isolated from the shoots and roots of wild plants. i.e.,*Amaranthusviridis*, *Ruelliatuberosa*, *Euphorbia hirta*, *Suaedafruticosa*, *Trichodesma amplexicaule* and *Haloxylon stocksii*. The biochemical characteristics of these strains showed that all 25 isolates of *P. fluorescens* were positive to catalase test, while negative for gelatin liquefaction and starch hydrolysis. The *Pseudomonas fluorescens* strains showed significant activity against root rotting fungi in dual plate assay, PGPR-HR1 showed the largest zone against *Fusarium oxysporum*of 24 mm, PGPR-HR3 effectuation was too impactful against *F. solani* produced zone of 24.25 mm, the growth of *Macrophomina phaseolina* was inhibited greatly by PGPR-HR1, PGPR-HR2 and PGPR-ES2 all produced zones of 23 mm, while the largest zone was produced by PGPR-AR1 and PGPR-HR3 both of 23.25 mm against *Rhizoctonia solani*, however fungal hyphae lysis was also occurred by some strains. *In vitro*, the strains of *P. fluorescens* were tremendously effective against root knot nematode by killing 2nd stage juveniles of *Meloidogyne incognita* at varying degrees. In screen house experiment, application of 8 potential strains of *P. fluorescens* viz. PGPR-AR1, PGPR-AR4, PGPR-HR1, PGPR-HR2, PGPR-HR3, PGPR-TR1, PGPR-SS1 and PGPR-HS1 rendered dexterous activity of bio-control against *M. phaseolina, F. oxysporum, F. solani, R. solani* and *Meloidogyne incognita*, the root knot nematodes. These strains also showed great impact on the plant growth by increasing length and weight of the chickpea plant.

Key-words: Antagonistic potential, Root-rotting fungi, fungal hyphae, Nematodes, Biocontrol activity, PGPR (plant growth promotion rhizobacteria)

INTRODUCTION

Chick pea (*Cicer arietinum* L.) ranked at third globally, after peas and beans (Kumar *et al*., 2007). It is produced in semi-arid tropical climates, with biotic and abiotic restrictions such as insects, barren soils, disease incidence, and climate variability limiting yield. Chickpeas are grown at a global rate of 968 kg ha-1. Chickpea production is low because of sensitivity to a variety of fungal, viral, and bacterial diseases. The yield losses in temperate zones ranges from 5 - 10 % while in tropical zones it ranges from 50-100%(Van Emden *et al*., 1988). Despite the usage of numerous molecular techniques and the use of inorganic fertilizers and pesticides concerning environmental circumstances over the last few decades, interest in environmentally friendly agricultural production and protection approaches has grown (Jannouraa*et al*., 2013). The environmentally-safe approach includes plant growth escalating microbes, and antagonistic microorganisms which reduce the perilous effects of synthetic fertilizers (Rupela*et al*., 2005; Khatoon*et al*., 2014).

Fluorescent pseudomonads have grouped into nonpathogenic saprophytic bacteria that colonize water, soil and plant surfaces (Shafique*et al*., 2015). It secretes a greenish fluorescent soluble pigment called pyoverdine which is a type of siderophore. Fluorescent *Pseudomonas* spp. producesiderophores in the soil that sequester the traces of Fe^{3+} making it unavailable to many pathogenic microorganisms present in the rhizosphere which is an essential element for their growth (Leong, 1986; Buysens*et al*., 1996).

Some strains of *Pseudomonas* spp. worked efficiently in the inhibition of growth and activities of plant parasitic nematodes (Cronin *et al*., 1997; Siddiqui andShaukat, 2003; Parveen*et al*., 2020) and also of several fungi and oomycete root pathogens (Raaijmakers*et al*., 2002). PGPR effectively control plant parasites and act as biological

control agents. A huge amount of studies is conducted on the relationship of *Pseudomonas* to soil suppressivness (Bettiol *et al*., 2009). Modifications in root exudates can prevent nematode eggs from hatching or lessen their attractiveness to plant roots (Becker *et al*., 1988).

The current study's purpose was to isolate *Pseudomonas* spp. from wild healthy plants and to determine their activity both in laboratory conditions and field conditions, and to determine their effectiveness as biological bio-control agent against phytopathogens.

MATERIALS AND MENTHODS

Plant samples collection for endophytic fluorescent *pseudomonas* isolation:

Different samples of wild plants i.e. *Amaranthus viridis* L., *Ruellia tuberosa* L., *Euphorbia hirta*, *Suaeda fruticosa*, *Trichodesma amplexicaule,* and *Haloxylon stocksii* were collected from the campus of the University of Karachi. In the laboratory the plant samples were placed at the low temperature till their use for isolation within24 h.

In vitro isolation of endophytic fluorescent *Pseudomonas*

One g roots and shoots of collected plants were taken from each sample and washed with tap water, then for about two to three minutes sterilized with 1% sodium hypochlorite and for about one minute again washed with distilled water. The shoots and roots then divided into small parts and blended with 50ml water which gives the dilution of 1:50. Then roots and shoot suspensions were prepared up to $1:10^5$ and $1:10^6$.1mL suspension of was placed in a petri dish which contained S1 medium (Manasa *et al.,* 2017).

The roots and shoots were then cut into very small pieces and 50mL water addition give the dilution of 1:50. Then shoots and roots suspension dilution were made up to $1:10^5$ and $1:10^6$ and transferred 1 mL suspension into S1 medium containing petri plates (Gould *et al*., 1985) which are treated with Trimethoprim treatment (Basham *et al*., 1993). Petri dishes were kept at 28 for three days of incubation. Under UV light at 366nm, florescent bacterial colonies seen, then for their purification King's B agar medium was used (King *et al*., 1954).

Gram Staining

A small amount of the culture of bacterial isolate was gently spread on the slide then air dried and for thrice over the flame, heat fixation was performed. A primary stain (crystal violet) is added about one minute and washed gently with stream of water, then blotted dry Lugol's iodine is then transferred to the slide for one minute for primary stain fixation and then washed under running water. The decolorizer (ethyl alcohol) was then added for about 30 seconds and washed. The secondary stain (safranin) was added to the bacterial smear for 1 minute, washed, dried, and observed with the help of microscope. Under the microscope, the appearance of purple to blue-black color indicated bacteria as gram-positive while the appearance of red color indicated bacteria as gram-negative.

Differentiation test among the fluorescent *Pseudomonas* species

Levan Formation Test

The bacterial growth appeared on nutrient agar supplemented with 5% sucrose (w/v) for the differentiation among the species of *Pseudomonas* i.e., *Pseudomonas aeruginosa. P. putida, P. fluorescens* have shown positive results, which exhibits nucleoid, convex colonies after three to five days of incubation at room temperature which indicates the formation of Levan test (Krieg and Holt, 1984), while negative results were shown by *P. putida and P. aeruginosa.*

Growth at 41°C

To differentiate among saprophytic fluorescent *Pseudomonas* the test bacterium was grown at 41°C. The performance was done to observe the *Pseudomonas aeruginosa* growth, if no growth so the test shows negative result (Krieg and Holt, 1984).

Growth at 4°C

The test bacterium was grown at 4°**C** to differentiate among the saprophytic fluorescent *Pseudomonas.* This test was performed to observe the *Pseudomonas fluorescence* growth, if bacteria show growth so the test shows positive results.

Biochemical Characterization

The standard procedures were followed for the biochemical characterization of bacterial isolates which may be described as below.

Catalase Test

Test cultures were inoculated on the slants of nutrient agar and kept for 24 hours incubation at 30°C. The cultures were supplemented with 3% hydrogen peroxide (H_2O_2). The test considered as positive for catalase activity by the production of gas bubbles (Schaad, 1992).

Gelatin Liquefaction

The test tubes containing sterilized nutrient gelatin were inoculated with PGPR cultures. Firstly, incubation period for test tubes was twenty four hours at 28 ± 2°C and incubation was done at 4°C then in refrigerator for thirty minutes. The results considered as positive, if test tubes remain liquefied and the test result was negative, if those that solidified on refrigeration (Blazevic and Ederer, 1975).

Starch Hydrolysis

Starch agar plates were inoculated with test cultures and left for twenty four hours of incubation at 28±2° C. The plates were supplemented with Lugol's iodine solution after incubation period and allowed to stand for fifteen to twenty minutes. The test for starch hydrolysis is considered as positive, if the clear zone is formed around the colony (Eckford, 1927).

Dual plate culture method (test against root-rotting fungi)

The technique for the determination of bacterial strains antifungal was introduced by Drapeau *et al*. (1973), the one side of petri plate is streaked with bacterial isolate which contains Czapek's-Dox Agar medium with 7.2 pH, and the 5mm diameter, disc of root-infecting fungus test was inoculated on the other side of the petri plate. The incubation for dishes were take place at 28°C and within three to seven days zone of inhibition (if any) was estimated.

Filtration of bacterial cell free culture

In the broth of KB, bacterial strains were grown at 30°C for forty-eight hours and twice centrifuged for fifteen minutes at 3000 rpm. The filtrate of the culture was collected in a beaker before use and the pellets were removed.

Root infecting fungi isolation from the soil

***Fusarium* spp. isolation by soil dilution technique**

Nine mL of 0.1% agar suspension was suspended with 1g of soil sample and dilution series were prepared. From the last dilution of soil of 0.1% of agar suspension 1mL aliquot was transferred over petri dishes which contain PCNB (Nash and Snyder, 1962) and the surface of agar was suspended with the suspension spread by dishes rotation. Plates kept for incubation for five days at 28°C and the species of *Fusarium* were examined by the reference to Booth (1971) and Nelson *et al*. (1983).

Isolataion of *Rhizoctonia solani* by baiting technique

After twenty four hours, the baits were removed and shifted on PDA at of 5.5 pH after washing these seeds under tap water for *Rhizoctonia solani* identification and growth (Wilhelm, 1995). The population of *Rhizoctonia solani* in soil can be estimated by seed colonization percentage.

Wet Sieving Technique and Dilution For *Macrohomina phaseolina* Isolation

Wet sieving technique was introduced as for the isolation of *Macrophomina phaseolina* by Sheikh and Ghaffar, (1975). Soil sample of 20g was wet sieved by the help of 100-mesh (150 µm) placed on 300 mesh (53 µm) screen. For about one minute, the residual obtained 53-µm was washed under tap water and relocated into a beaker which contains 0.5% $Ca(OCl)_2$ and made up to 100mL for 1:5 dilution formation. The suspension of sclerotia was placed on a magnetic stirrer and aliquot of 1mL was evenly spread onto PDA plates surface containing penicillin (100, 000 units/ L) and demosan (0.3 g/L), streptomycin (0.2g/L) and rose Bengal 0.1 g/L. The incubation period of plated were five days at 28°C and grayish to black *Macrophomina phaseolina* colonies were observed. .

Identification of Nematodes Species

For the collection of the root-knot nematodes, plant roots Infected with nematodes were collected from Karachi University. Under a stereomicroscope, the washing and dissection of infected roots was performed to obtain females. Perennial patterns help as explained by Taylor and Sasser (1978) in identification of *Meloidogyne incognita.*

Root-Knot Eggs Extraction

Roots infected with nematodes were cut into small pieces and added to the solution of 1% sodium hypochlorite in a bottle, the bottle was tightly sealed. For about three minutes, the bottle was shaken vigorously manually, then contents were transferred onto a sieve of 100-mesh, placed over a sieve of mesh size 40. The sample was washed under tap water for 12 min and the collected residual on a sieve of 400-mesh was shifted into a beaker of 2500mL. Counting chamber helped in estimating number of juveniles/eggs per mL of suspension (Hussey and Barker, 1973).

Juvenile's Mortality of Root-Knot Nematode

Two mL culture filtrate of test bacterium and 1mL suspension of freshly hatched stage juvenile was shifted in glass cavity block at 28° C. In the glass cavity block, 2mL of KB broth is present which didn't have bacterium cultural filtrate, served as control. Each treatment has three replicates and estimation of juvenile mortality was done after twenty four hours and forty eight hours.

***In Vivo* Screen House Experiment**

Each earthen pot containing 1000 g of soil was suspended with 20mL freshly hatched stage juvenile suspension. Bacterial cultural cell suspension which was five-day-old was drenched in pots of eight strains of test bacteria that were used. In this experiment, along with the control pots, the fungicide (Carbendazim) treatment was also used. In each pot eight were sown, and only four seedlings were kept per pot after germination. The seedlings were uprooted after forty-five days, fresh length and weight of shoots and roots were taken, root-knot nematode and root-infecting fungi infection were also checked.

Calculation of infection percentage as below:

$$\text{Infection \%} = \frac{\text{Total Number of plant roots infected by a pathogen}}{\text{Total number of plant roots}} \times 100$$

The number of root knots was counted to determine the infection of nematodes on the root system.

Experimental Design

Plant growth promoting bacteria (PGPR) used in performing experiments, as a soil drench in controlling root-knot nematode and root infecting fungi on the crop of chickpea. There were four replicates for each treatment and pots were recombined under the Biological Research center's green-house, University of Karachi.

Analysis of Data

Analysis of data was performed and subjected to variance analysis (ANOVA) and by using least significant difference (LSD), means were separated suggested by Gomez and Gomez (1984).

RESULTS

***In-vitro* isolation of endophytic fluorescent *Pseudomonas* spp.**

Twenty five strains of fluorescent *Pseudomonas* were isolated and identified from the roots and shoots of wild plants that were collected from Department of Islamic Learning, University of Karachi (Table 1).

Tests for differentiation among the species and biochemical tests for the characterization of fluorescent *Pseudomonas*

Among the 25 isolates of fluorescent *Pseudomonas* all the isolates were differentiated as *Pseudomonas fluorescens* as all the test bacteria grew on Nutrient agar added with 5% sucrose. They showed positive results exhibiting convex, mucoid colonies after incubation of 2-3 days which shows positive Levan formation test (Krieg and Holt, 1984). And no strain was grown at 41°C while strains showed growth at 4°C (Table 2).

All the test PGPR cultures showed positive results for catalase test as they produced gas bubbles on the addition of 3% hydrogen peroxide (H_2O_2). However showed negative results for starch hydrolysis and gelatin liquefaction (Table 2).

Table 1.*Pseudomonas fluorescens* strains with host name, plant part, locality, and strain name.

Bacterial strains	**Locality**	**Name of host Plant**	**Plant part**	**Species Isolated**
PGPR-AR1	Islamic learning department (UOK)	*Amaranthus viridis*	Root	*Pseudomonas fluorescens*
PGPR-AR3	Islamic learning department (UOK)	*Amaranthus viridis*	Root	*Pseudomonas fluorescens*
PGPR-AR4	Islamic learning department (UOK)	*Amaranthus viridis*	Root	*Pseudomonas fluorescens*
PGPR-AS1	Islamic learning department (UOK)	*Amaranthus viridis*	Shoot	*Pseudomonas fluorescens*
PGPR-AS2	Islamic learning department (UOK)	*Amaranthus viridis*	Shoot	*Pseudomonas fluorescens*
PGPR-AS3	Islamic learning department (UOK)	*Amaranthus viridis*	Shoot	*Pseudomonas fluorescens*
PGPR-AS4	Islamic learning department (UOK)	*Amaranthus viridis*	Shoot	*Pseudomonas fluorescens*
PGPR-RS1	Islamic learning department (UOK)	*Rullia tuberosa*	Shoot	*Pseudomonas fluorescens*
PGPR-RS3	Islamic learning department (UOK)	*Rullia tuberosa*	Root	*Pseudomonas fluorescens*
PGPR-HR1	Islamic learning department (UOK)	*Haloxylon stocksii*	Root	*Pseudomonas fluorescens*
PGPR-HR2	Islamic learning department (UOK)	*Haloxylon stocksii*	Root	*Pseudomonas fluorescens*
PGPR-HR3	Islamic learning department (UOK)	*Haloxylon stocksii*	Root	*Pseudomonas fluorescens*
PGPR-HR4	Islamic learning department (UOK)	*Haloxylon stocksii*	Root	*Pseudomonas fluorescens*
PGPR-HR5	Islamic learning department (UOK)	*Haloxylon stocksii*	Root	*Pseudomonas fluorescens*
PGPR-HS1	Islamic learning department (UOK)	*Haloxylon stocksii*	Shoot	*Pseudomonas fluorescens*
PGPR-TR1	Islamic learning department (UOK)	*Trichodesma amplexicaule*	Root	*Pseudomonas fluorescens*
PGPR-TR2	Islamic learning department (UOK)	*Trichodesma amplexicaule*	Root	*Pseudomonas fluorescens*
PGPR-SS1	Islamic learning department (UOK)	*Suaeda fruticosa*	Shoot	*Pseudomonas fluorescens*
PGPR-ES2	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*
PGPR-ES3	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*
PGPR-ES4	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*
PGPR-ES5	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*
PGPR-ES6	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*
PGPR-ES7	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*
PGPR-ES8	Islamic learning department (UOK)	*Euphorbia hirta*	Shoot	*Pseudomonas fluorescens*

Anti-fungal activity of bacterial strains

The antagonistic activity of 25 different strains of fluorescent *Pseudomonas* against the 4 root rotting fungi i.e. *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* were examined in dual culture plate method (Table 3). PGPR-HR1, PGPR-HR2, PGPR-HS1, PGPR-AR1, PGPR-HR3, PGPR-TR1, PGPR-SS1 and PGPR-ES4 showed maximum inhibition of *Fusarium oxysporum* by producing zones of 24 mm, 22 mm, 21.75 mm, 21.5 mm, and 21.75 mm respectively. The best results against *Fusarium solani* was shown by PGPR-AR1 (23.75 mm), PGPR-HR2 (22.25 mm), PGPR-HR3 (24.25 mm), PGPR-TR1 (22.25 mm), PGPR-SS1 (21.25 mm), PGPR-ES4 (21.25 mm), PGPR-ES7 (22.25 mm) and PGPR-ES8 (23 mm). While maximum suppression of *Macrophomina phaseolina* was shown by PGPR-HR1, PGPR-HR2, PGPR-HS1, PGPR-SS1, PGPR-ES2 and PGPR-ES4 by producing zones of inhibition of 23 mm, 23 mm, 22.75 mm, 22.5 mm, 23 mm and 22.25 mm, respectively. And PGPR-AR1, PGPR-HR2, PGPR-HR3, PGPR-HS1, PGPR-ES3, PGPR-ES5 and PGPR-ES6 showed best results against *Rhizoctonia solani* by inhibiting its growth and making zones of 23.25 mm, 22.75 mm, 23.25 mm, 22.75 mm, 22.5 mm, 21.75 mm and 21.5 mm, respectively.

Table 2. Tests for differentiation among the species and biochemical tests for the characterization of fluorescent *Pseudomonas.*

Bacterial Strains	**Growth at 25°C**	**Growth at 41°C**	**Growth at 4°C**	**Catalase Test**	**Gelatin Liquefaction**	**Starch Hydrolysis**
PGPR-AR1	+	-	+	+	-	-
PGPR-AR3	+	-	+	+	-	-
PGPR-AR4	+	-	+	+	-	-
PGPR-AS1	+	-	+	+	-	-
PGPR-AS2	+	-	+	+	-	-
PGPR-AS3	+	-	+	+	-	-
PGPR-AS4	+	-	+	+	-	-
PGPR-RS1	+	-	+	+	-	-
PGPR-RS3	+	-	+	+	-	-
PGPR-HR1	+	-	+	+	-	-
PGPR-HR2	+	-	+	+	-	-
PGPR-HR3	+	-	+	+	-	-
PGPR-HR4	+	-	+	+	-	-
PGPR-HR5	+	-	+	+	-	-
PGPR-HS1	+	-	+	+	-	-
PGPR-TR1	+	-	+	+	-	-
PGPR-TR2	+	-	+	+	-	-
PGPR-SS1	+	-	+	+	-	-
PGPR-ES2	+	-	+	+	-	-
PGPR-ES3	+	-	+	+	-	-
PGPR-ES4	+	-	+	+	-	-
PGPR-ES5	+	-	+	+	-	-
PGPR-ES6	+	-	+	+	-	-
PGPR-ES7	+	-	+	+	-	-
PGPR-ES8	+	-	+	+	-	-

Mortality of root knot nematodes (juvenile's)

Cell free culture filtrates of fluorescent *Pseudomonas* strains showed significant ($p<0.05$) nematicidal effects by killing second stage juveniles at varying degrees (Table 4). Maximum killing after 24 hours was observed by PGPR-AS1, PGPR-RS1, PGPR-HR4, PGPR-HR5, PGPR-SS1, PGPR-ES2, PGPR-ES3, PGPR-ES4, PGPR-ES5 and PGPR-ES8, all strains kill juveniles at 53.33 % and after 48 hours maximum juveniles were killed by PGPR-AR1, PGPR-AS2, PGPR-AS4, PGPR-RS3, PGPR-HR2, PGPR-HS1, PGPR-TR2, PGPR-SS1, PGPR-ES6, PGPR-ES7 at 100 %.

***In vivo* effect of endophytic fluorescent *pseudomonas* on chick pea in screen house experiment**

This experiment was performed to observe the growth of chickpea plant i.e. shoot/root length, fresh weight of shoot and root, infection percentage of root-infecting fungi and number of root knots after 45 days of nematode inoculation.

PGPR-SS1, PGPR-TR1 and PGPR-HR2 showed maximum shoot length of 21.39 cm, 20.48 cm and 20.74 cm, respectively. Maximum root length was shown by PGPR-HR2 (19.23 cm), PGPR-AR1 (18.61 cm), PGPR-HR1 (18.65 cm) and PGPR-SS1 (18.25 cm). The maximum shoot weight was observed in plants of strains PGPR-AR1 (2.92 g), PGPR-HR1 (2.41 g) and PGPR-TR1 (2.61 g). Whereas maximum root weight was observed in plants of

strains PGPR-AR1, PGPR-AR4 and PGPR-HR1 with root weight of 1.40 g, 1.16 g and 1.13 g, respectively (Table 5).

Maximum number of nematode knots in roots of chickpea plant was significantly ($p<0.05$) decreased by PGPR-HR1 (0.37), PGPR-SS1 (0.31) and PGPR-HS1 (0.31) (Table 5).

The root-infecting fungi were significantly ($p<0.05$) reduced by the following strains (**Figure 1**). The infection of *Fusarium oxysporum* was controlled greatly by PGPR-AR1 (10%) and PGPR-TR1 (11.25%). PGPR-AR1, PGPR-HR3 and PGPR-TR1 showed great control of *Fusarium solani* by 7.5%, 6.25% and 8.75%, respectively. The infection of *Macrophomina phaseolina* was maximum reduced by PGPR-HR1 (1.25%), PGPR-TR1 (2.5 %), PGPR-AR1 (3.75%) and PGPR-HR3 (3.75%). Whereas, the infection of *Rhizoctonia solani* was greatly reduced by PGPR-HR1 (16.25%), PGPR-HR2 (22.5%) and PGPR-HS1 (23.75%).

Table 3. *In vitro* inhibition of *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* by strains of fluorescent *Pseudomonas.*

Bacterial Strains	***F. oxysporum* (mm)**	***F. solani* (mm)**	***M. phaseolina* (mm)**	***R. solani* (mm)**
PGPR-AR1	21.75	23.75	21.75	23.25
PGPR-AR3	20.75	20.5	19.5	20.75
PGPR-AR4	21	21.5	21	20
PGPR-AS1	18.5	20	19.25	*
PGPR-AS2	19.25	**	17.75	19.75
PGPR-AS3	20.5	*	19	20
PGPR-AS4	19.25	20	**	19.25
PGPR-RS1	20.5	***	18	19
PGPR-RS3	18	19	***	18
PGPR-HR1	24	21	23	20.5
PGPR-HR2	22	22.25	23	22.75
PGPR-HR3	21.5	24.25	21	23.25
PGPR-HR4	***	19.5	18.75	15.25
PGPR-HR5	***	19.75	16	20
PGPR-HS1	22	20.25	22.75	22.75
PGPR-TR1	21.5	22.25	20.75	20.5
PGPR-TR2	20	20.25	*	19
PGPR-SS1	21.5	21.25	22.5	20.25
PGPR-ES2	19	20	23	21
PGPR-ES3	*	20	20.5	22.5
PGPR-ES4	21.75	21.25	22.25	20.5
PGPR-ES5	19	***	19	21.75
PGPR-ES6	18.75	20.25	21.5	21.5
PGPR-ES7	20.75	22.25	20.75	*
PGPR-ES8	20.5	23	20.25	18.75

*- No Zone of Inhibition; **- Colonies met each other and fungal mycelium lysed; ***- Test fungus over grew on the bacterium

Table 4. Effects of the cell free culture filtrates of different strains of *Pseudomonas* on juvenile's mortality of *Meloidogyne incognita* after 24 and 48 hours.

Bacterial Strains	**24 hours (%)**	**48 hours (%)**
Control (KB Broth)	0	0
PGPR-AR1	46.66	100
PGPR-AR3	33.33	93.33
PGPR-AR4	40	93.33
PGPR-AS1	53.33	93.33
PGPR-AS2	46.66	100
PGPR-AS3	33.33	86.66
PGPR-AS4	33.33	100
PGPR-RS1	53.33	86.66
PGPR-RS3	33.33	100
PGPR-HR1	46.66	93.33
PGPR-HR2	40	100
PGPR-HR3	46.66	93.33
PGPR-HR4	53.33	93.33
PGPR-HR5	53.33	93.33
PGPR-HS1	40	100
PGPR-TR1	46.66	93.33
PGPR-TR2	46.66	100
PGPR-SS1	53.33	100
PGPR-ES2	53.33	93.33
PGPR-ES3	53.33	93.33
PGPR-ES4	53.33	93.33
PGPR-ES5	53.33	86.66
PGPR-ES6	46.66	100
PGPR-ES7	40	100
PGPR-ES8	53.33	93.33
LSD0.05	19.27[1]	14.36[1]

[1]In column, mean values presenting differences more than LSD value at $p<0.05$ are significantly different.

Table 5. Effects of endophytic fluorescent *Pseudomonas* and carbendazim on the growth of chickpea plant.

Treatments	**Shoot Length**	**Shoot Weight**	**Root Length**	**Root Weight**	**Number of Knots**
	(cm)	**(g)**	**(cm)**	**(g)**	
Control	16.68	1.82	10.13	0.32	1.25
Carbendazim	20.25	2.04	17.88	0.54	0.56
PGPR-AR1	19.82	2.92	18.61	1.4	0.75
PGPR-AR4	15.81	1.62	15.22	1.16	0.62
PGPR-HR1	19.38	2.41	18.65	1.13	0.37
PGPR-HR2	20.74	2.17	19.23	1.08	0.5
PGPR-HR3	19.46	2.36	14.8	0.57	0.68
PGPR-TR1	20.48	2.69	17.7	1.08	0.62
PGPR-SS1	21.39	2.28	18.25	0.69	0.31
PGPR-HS1	15.71	2.01	13.76	0.92	0.31
$LSD_{0.05}$	1.57[1]	0.76[1]	2.75[1]	0.38[1]	0.5[1]

[1]In column, mean values presenting differences more than LSD value at $p<0.05$ are significantly different.

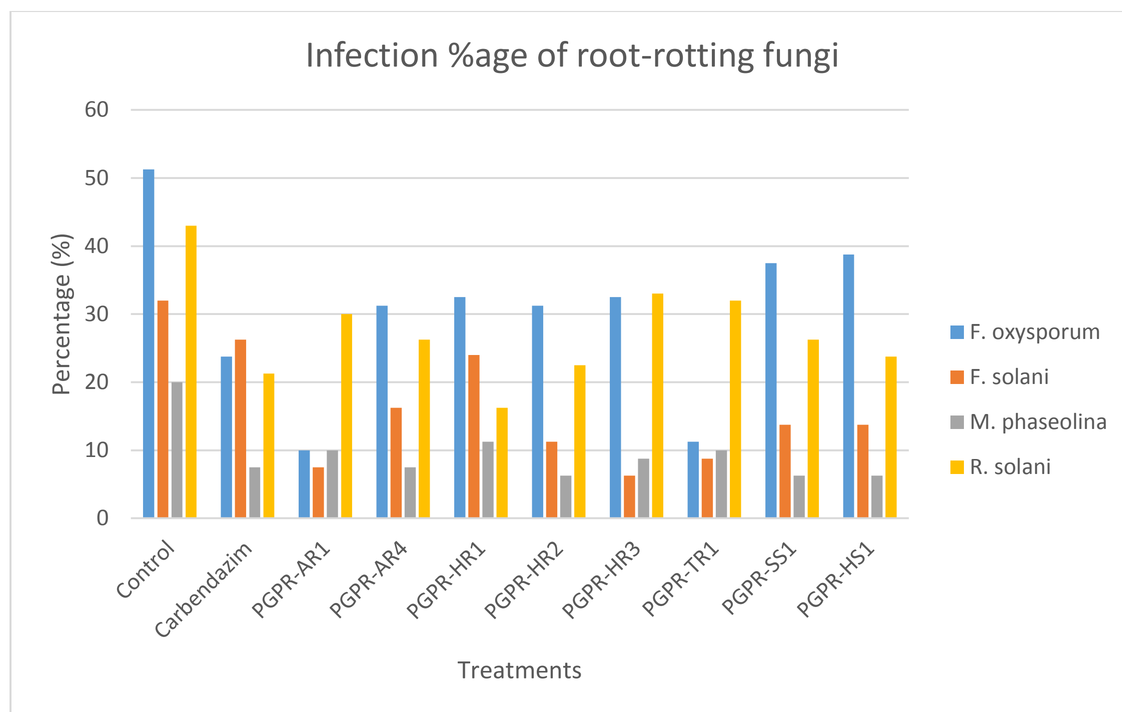

Fig. 1. Effects of endophytic fluorescent *Pseudomonas* and carbendazim as soil drench on the infection of *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina,* and *Rhizoctonia solani* on chickpea roots.

DISCUSSION

In this present investigation 25 strains of fluorescent *Pseudomonas* were isolated from the roots and shoots of different wild plants viz. *Amaranthus viridis, Ruellia tuberosa, Euphorbia hirta*, *Suaeda fruticosa, Trichodesma amplexicaule*, and *Haloxylon stocksii*, and the effect of these strains was observed *in-vitro* and *in-vivo* against root-infecting fungi and root-knot nematodes. Pseudomonads are responsible in improving growth of plant by mitigating the proliferation of root infecting pathogens and also produce biologically active compounds (Oostendorp and Sikora, 1989; Gamliel and Katan, 1993). Pseudomonads are also capable of converting unavailable minerals into forms which plant can uptake easily (Broadbent *et al*., 1977; Siddiqui and Mahmood, 1999). Several species of *Pseudomonas* can synthesize enzymes that can regulate plant hormone levels, may limit the available iron by the production of siderophores and can also kill the pathogen with antibiotics (Siddiqui, 2006).

In this study, tests for differentiation among the species and biochemical tests for the characterization of fluorescent *Pseudomonas* bacteria were carried out. All 25 isolates were able to grow at room temperature, no strain was grown at 41°C, and all grown at 4°C. All of the isolates showed positive levan formation test which showed that all of the strains belongs to *Pseudomonas fluorescens.* All the isolates produced gas bubbles on the addition of 3% hydrogen peroxide (H_2O_2) in the catalase test, giving positive results (Schaad, 1992). Similar results were shown by Saravanan *et al*., (2013), all the isolates were positive to catalase activity. All strains showed negative results in gelatin liquefaction and starch hydrolysis test, similar results were reported by Nepali *et al*. (2018).

In dual culture plate method, *Pseudomonas* strains PGPR-HR1, PGPR-HR2 and PGPR-HS1 were found to be most effective against *Fusarium oxysporum* in dual plate assay. According to Muhammed *et al*. (2011), the effect of *Pseudomonas* strain against *Fusarium oxysporum* was also found to be effective and the growth of *F. oxysporum* was limited to 50% in dual culture plate method. In case of *Rhizoctonia solani*, PGPR-AR1 and PGPR-HR3 showed greater zones of inhibition. Maurya *et al*. (2014) described their results of fluorescent *Pseudomonas* strains which were quite similar against *Rhizoctoniasolani*. Strains PGPR-HR1, PGPR-HR2 and PGPR-ES2 showed best results against *Macrophomina phaseolina*. In previous study by Devi *et al*. (2011), fifteen fluorescent *Pseudomonas* strains were able to inhibit *M. phaseolina* in dual plate assay. The best results against *Fusariumsolani*was greatly inhibited by PGPR-AR1 and PGPR-ES8 producing maximum zones of inhibition. Palani *et al*. (2015) showed in their study, *P. fluorescens* strain inhibited growth of *F. solani* at maximum level.

Pots treated with fluorescent *Pseudomonas* exhibit a significant increase in root and shoot length and weight over the untreated control and Carbendazim. Strains PGPR-SS1, PGPR-TR1 and PGPR-HR2 increased the length of shoot at maximum level. For shoot weight the best results were shown by PGPR-AR1 and PGPR-TR1. The highest root length was shown by strains PGPR-HR2, PGPR-HR1 and PGPR-AR1. Root weight was increased maximum by PGPR-AR1, PGPR-AR4 and PGPR-HR1. According to Kumar *et al*., (2007), chickpea plant treated with fluorescent *Pseudomonas* showed maximum increase in plant height as compared to the untreated plant by strain Pf4-99. Saravanan *et al.* (2013) also described in their results, by inoculation with fluorescent *Pseudomonas* there was a massive increase in roots and shoots length of plants as compared to the un-inoculated control in tomato plant. However, in this study Carbendazim showed not much better control against root infecting fungi as well as root knot nematodes, also it did not increase massive length and weight of the plants. Similar results were shown by Kumar *et al.* (2007) and Rubina *et al.* (2018), in which no prominent increment in the height of plants treated with carbendazim.

The usage of *Pseudomonas fluorescence* improves the cell wall structure of host and it limits the entry of pathogen into the plant tissues (Benhamou *et al*., 2000; Chen *et al*., 2000; Conrath *et al*., 2002; Dwivedi and Johri, 2003). In this study, fluorescent *Pseudomonas* play the similar role by controlling root rotting pathogens from entering the host plant present in the soil. The significant ($p<0.05$) results showed that *Macrophomina phaseolina* was controlled maximum as compared to the other root rotting fungi viz. *Fusariumsolani, Fusarium oxysporum* and *Rhizoctonia solani.* However, a significant reduction in disease severity was also observed in other root-infecting fungi. Comparable results were described by Izhar *et al*. (1995) in which different strains of fluorescent *Pseudomonas* were found to be significant ($p<0.05$) in controlling the infection of *M. phaseolina*, *R. solani* and *Fusarium* spp. on chickpea.

Present study shows*, in vitro* nematicidal activity of endophytic fluorescent *Pseudomonas* was observed significant ($p<0.05$) by killing second stage larvae of *Meloidogyne incognita.* Several fluorescent *Pseudomonas* strains were highly effective against *Meloidogyne incognita* by killing its second stage juveniles (Wahla *et al*., 2012). *In vivo* studies also showed significant results against root knot nematodes by the reduction of forming galls or knots in roots of chickpea plant (Table 4). Maximum reduction of galling of *Meloidogyne incognita* was also observed in plants treated with endophytic fluorescent *Pseudomonas* strains (Singh and Siddiqui, 2010).

In this study, the bacterial strains of *Pseudomonas fluorescens* exhibited significant activity against root-rot causing fungi and root knot nematode both *in vitro* and *in vivo*. There are reports that PGPR are show mutual interaction with the host plants (Pandey *et al*., 2005). They can either directly or indirectly boost plant development by creating phyto-hormones, biocontrolling host plant diseases, or improving plant nutritional status (Glick, 1995). Endoparasitic nematodes, such as *Meloidogyne* spp., establish specialized feeding cells in plant tissue and remain entrenched in it. Endophytic PGPR invading plant root tissue may thus be more capable of managing endo-parasitic nematodes and fungus. The use of endophytic microorganisms for the control of root-rot causing fungi and parasitic nematodes is a relatively new approach and the use of PGPR for control of soil borne diseases is better because they provide protection to plant roots as well as shoots from parasitic pathogens.

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