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BIOCONTROL POTENTIAL OF PSEUDOMONAS AERUGINOSA STRAINS AGAINST ROOT ROT PHYTOPATHOGENS

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Abstract

Each year billions of dollars in losses occur due to root rot pathogens which affect almost all types of plants and it is burning issues for the plants world. As these pathogens invade the roots of the plants which ultimately destroy the whole plants. The use of microorganisms is environment friendly, escalated global food production with obscure environmental hazards and has a strong potential to destroy these root rot pathogens while enhancing plant immunity. Keeping this in mind, wild healthy plants from different areas of Karachi were collected for the isolation of endophytic *Pseudomonas aeruginosa.* In this study, total 14 strains of fluorescent *Pseudomonas* were isolated from plant samples in order to check their potentially initially *In vitro* activity and then potential strains were applied in pot experiments. *In vitro* activity, *P. aeruginosa* strains showed noteworthy activity contrary to root rotting fungi in dual plate assay. Culture filtrates of *P. aeruginosa strains* showed strong antimicrobial activity against some common lab bacteria viz., *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli* by formation of prominent zone of inhibition. In the screen house experiment, application of all 14 strains of *Pseudomonas aeruginosa* showed significant bio-control activity against four major root rotting fungi viz., *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani* and *Macrophomina phaseolina.* Significant changes were observed in the amount of chlorophyll and carotenoid in mung bean plants that were inoculated with various strains. Significant increase in the amount of carbohydrates and proteins showed that these strains have strong potential to boost the immunity of plants and can be further used for agriculture. It is thus revealed that a multifunctional PGPR acts as a potential biocontrol agent to control fungal and bacterial pathogens.

Key words: *Pseudomonas aeruginosa, Rhizoctonia solani, Fusarium oxysporum, F. solani, Macrophomina phaseolina*.

Introduction

Mung bean is a rich source of Fe (5.9-7.6 mg/100 g) and protein (14.6-33.0 g/ 100 g). The color of grain is associated with compounds like carotenoids and polyphenols, whereas the fiber content gives hardness to grain. Dahiya *et al*., (2015) reported that Phytic acid, tannins, hemagglutinins, and polyphenols are present in mung beans. In Pakistan, Mung bean is grown on the largest pulse area following chickpea as the first. It is grown in both rain fed and in irrigated areas. Because it is a short-term crop, it requires less water than summer crops (Anjum *et al*., 2006). An enhanced production is essential to provide an adequate amount of food for the growing human population. Root rotting pathogens are prevalent in the soil and can cause devastating impacts on the crops and properties of soil. According to Grewal (1988) the major fungi responsible for root rot of mung beans include *Rhizoctonia solani*, *Fusarium* spp. and *Macrophomina phaseolina. Macrophomina phaseolina* cause rot of seedlings, collar, pod, and blight of leaves (Sadhu & Singh, 1998). Wet rot in mung beans is caused by *Rhizoctonia solani* (Dubey *et al*., 2011). Fusarium wilt which affects crops severely is caused due to *Fusarium* spp. (Sun *et al*., 2019). *Fusarium* spp. blocks the vascular tissues of infected plant parts and cause wilt which ends up with the death of tissues. Crop Rotation System of Mung bean with other crops increases both land and crop productivity particularly and is important for sustainable agriculture. Annual crop legumes grown in rotation with cereals contribute to total amount of nitrogen in the soil and improved yield of cereals (Ahmad *et al*., 2001).

The first definition of an endophyte, provided by De bary (1866) is as any organism that grows within plant tissues are termed as endophytes, however, according to different scholars, the definition keeps changing. Endophytes are the type of microbes that may penetrate plant hosts and colonize intercellular spaces. They colonize the plant systemically, found in a wide range of plant tissues, along with the bacterial and fungal colonies (Jalgaonwala *et al*., 2011). Endophytic bacteria occur naturally in healthy plant organs, develop a relationship with the host plant without causing any harm to it, and are generally not considered as pathogens (Mano & Morisaki, 2008). The growth and health of a plant is promoted by endophytic bacteria, and these mechanisms are performed as a result of the production of phytohormones and enzymes responsible for the growth regulator metabolism (Taghavi *et al*., 2009). Endophytic bacteria stimulate plant growth by producing siderophores which are low molecular weight Fe-chelating compounds, or solubilize phosphate and ACC deaminase (Rajkumar *et al*., 2009).

The community of microbes present in the rhizospheric niche produce various chemicals, which is how PGPR-mediated escalated growth of plants occur (Kloepper *et al*., 1980). In general, PGPR escalate the growth of plants either directly (by aiding the acquiring of N, P, and important minerals) or indirectly (by minimizing the deleterious impacts of phytopathogens on the development and proliferation of plants) (Glick, 2012).

Similar to pathogen-induced systemic acquired resistance (SAR), induced resistance mediated by rhizobacteria increases the resistance of uninfected plant sections to plant diseases such viruses, bacteria, and fungi and also to nematodes and insects. (Bent, 2006; Pozo & Azcon-aguilar, 2007). In the same plant, the same strain causes resistance to a variety of diseases (Somers *et al*., 2004). A lot of research work has been carried out on species of *Pseudomonas* and *Bacillus* for their induced systemic resistance in plants (Van wees *et al*., 2008). Irrespective of the signalling operations used, De-Vleesschauwer & Hofte (2009) adopted the term ISR to describe induced systemic resistance caused by non-pathogenic bacteria or PGPR, whereby SAR is the result of localized infection and it is salicylic-acid dependent induced resistance. The present study aim is to isolate endophytic *Pseudomonas* strains and to check their efficacy for the potential application for the better growth and disease suppression in mung beans.

Material and Methods

Isolation of endophytic fluorescent *pseudomonas (PGPR*): Samples of different wild plants i.e. *Aerva javanica, Cressa cretica, Datura alba, Euphorbia hirta*, *Enicostemma verticillatum*, *Phragmites australis, Pulchea indica, Rhyncosia minima, Suaeda nudiflora, Senna holosericea, Trichodesma idicum* were randomly collected from the field of University of Karachi (Kathor **Latitude:** 25.03298; **Longitude:** 67.37588). The land area were having pH 6.8 and soil were sandy loam. Plants were transported to the lab and stored there for 24 hours at an extremely low temperature before being used for isolation. 1g roots and shoots of collected plants were taken from each sample and washed away with running water, disinfected with 1% sodium hypochlorite (NaOCl) for 3 minutes and washed away again with sterilized water for 1 minute to reduce the effect of NaOCl. The roots and shoots were then cut into minute pieces and blended with 50 mL H_2O (1:50) dilution. Then the dilutions of the plant samples were prepared up to $1:10^5$ and $1:10^6$ and were transferred in the petri plate (1 mL) having S1 medium (Gould *et al*., 1985). Petri plates were kept at 25℃ for 2 days. Under UV light at 366 nm, bacterial colonies that appear fluorescent were taken with a sterilized wire loop and purified on King's B medium.

Test for species identification of fluorescent *Pseudomonas*

Levan formation test: The bacterial strains were grown on nutrient agar medium accompanied with 5 percent of sucrose to differentiate among the *pseudomonas* species i.e. *Pseudomonas fluorescens*, *P. putida* and *P. aeruginosa*. *Pseudomonas fluorescens* showed positive results, exhibiting convex, mucoid colonies after incubation of 48-72 hours at room temperature that reveals the levan formation (Krieg & Holt, 1984), while *P. putida* and *P. aeruginosa* exhibited negative results.

Growth at 41°C: Differentiation among the saprophytic fluorescent *Pseudomonas* was done via growing the test bacterium at 41°C. This test was done to observe the growth of *Pseudomonas aeruginosa,* if not grown then the test is negative (Krieg & Holt, 1984).

Biochemical characterization of bacteria: The biochemical testing of the bacterial strains was followed using standard procedures as detailed below.

Starch hydrolysis: On the starch agar plates, the test cultures were inoculated and left to grow for a day at room temperature. The plates were saturated with Lugol's iodine and left to stand for ten–twenty minutes at room temperature. It was thought that the creation of a clean zone around the colony indicated successful hydrolysis of starch. *Pseudomonas aeruginosa* shows negative results (Lal & Cheeptham, 2012).

Cell-free filtrate of bacteria: The isolates of bacteria were inoculated on King's B broth and kept at room temperature for 2 days, centrifuged for 15 mins at 3000 rpm, and the supernatant was collected in a sterilized test tube while the pallet was discarded.

Dual culture plate method: This procedure is carried out to evaluate the activity of isolated bacteria against the fungal pathogens. For this purpose, the freshly grown bacterium was streaked on one end, while a 5 mm disc of the fungal pathogen (*Fusarium solani, Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium oxysporum*) was kept on another end of the Petri plate containing Czapek's dox agar. The plates were kept at 25℃ for 5-7 days and the inhibition zone was measured (Farhat *et al*., 2017).

Disc diffusion method: The activity of bacterial strains against the common lab bacteria was determined according to disc-diffusion method, shortly 5 mm disc of sterilized filter paper was loaded with the bacterial filtrate i.e., 20, 40 and 60 µL per disc and placed overnight in a clean biological safety cabinet. Bacterial lawn of common lab bacteria, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium* were prepared on nutrient agar plates. Streptomycin and penicillin (20µg/disc) served as positive control (loaded and placed overnight in a clean biological safety cabinet), while sterilized distilled water served as negative control. The discs were placed in clockwise direction on a nutrient agar plate containing a lawn of bacteria, starting with negative control, positive control, 20, 40, and 60 µL and kept incubated 48 hours at room temperature. The zone of inhibition around the disc was measured in mm (Farhat *et al*., 2019; 2022).

Experimental design: The PGPR were drenched in the soil in order to mitigate the proliferation of root deteriorating fungi on mung bean crop in a screen house experiment. Each treatment was replicated 4 times, and pots were placed in randomized block design on a greenhouse bench of biological research Center, university of Karachi (Farhat *et al*., 2023).

Screen house experiment: 14-day old bacterial culture cell suspension was drenched in pots of all 14 strains of test bacteria that were used. One treatment of fungicide (topsin) was used in this experiment along with the control pots. Seven seeds were sown in each pot and water them on the basis of requirement, after sprouting only four seedlings were kept per pot. Natural light were available for plants and pots were kept under green sheds. After 45 days, the seedlings were uprooted, fresh weight and length of roots and shoots were taken, and infection of root-infecting fungi was examined (Urooj *et al*., 2021; Farhat *et al*., 2023).

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

Biochemical parameters

Chlorophyll and carotenoid: Chlorophyll and carotenoid were extracted with 80% aqueous acetone, 1 g of leaf sample was crushed in 5 mL 80% acetone, centrifuged at 4500 rpm for 10 minutes, and supernatant was collected in another tube. For estimated OD was taken at 663, 645, 500 and 410 nm, 80% acetone was treated as blank in spectrophotometer Lichtenthaler & Wellburn (1983).

Carbohydrate: Estimation of carbohydrate from treated and control plant samples was done by anthrone reagent using the method of Yemm & Willis (1954). Using distilled water 0.5 g of sample was homogenized at 500 rpm for 5 minutes, clear supernatant was collected in test tube. 1 ml of supernatant was added with 5ml of anthrone, boiled for 30 minutes and cooled with cold water, OD was measured at 620 nm and anthrone reagent was kept blank.

Protein: For the amount of protein content, 0.5 g of sample with 5mL of distilled water was homogenized and for 10 minutes centrifuged at 3000 rpm. Supernatant was collected in a test tube. 0.5 mL of supernatant was added with 3 mL of Alkaline copper solution (formed by mixing 50 mL of Sodium Carbonate 2% in 0.1N NaOH and 1 mL of CuSO4 0.5% in 1% Sodium Potassium tartrate), incubate for 10 minutes at 25℃, 1 mL of Folin reagent was added and OD was measured at 750 nm (Lowry *et al*., 1951).

Analysis of data: Statistical evaluation amongst all treatments for above and below ground dry plant biomass and proportion assistance thereof to overall dry plant biomass was conducted using one-way analysis of variance (ANOVA) with stiffens post hoc test. Assumptions of all tests were examined and statistically verified. All statistical analysis was carried out using SPSS 10.0 (SPSS Inc, USA) for windows.

Results and Discussion

Isolation of endophytic fluorescent *Pseudomonas*: 14 isolates of *Pseudomonas* were extricated and identified from the roots and shoots of wild plants that were collected from University of Karachi. The identified and experimentally used *Pseudomonas* strains were then submitted to Karachi University Culture Collection Center in order to get accession numbers. The accession number is provided for each strains *viz*; PGPR-A (KUCC1387), PGPR-B (KUCC1388), PGPR-C (KUCC1389), PGPR-D (KUCC1390), PGPR-E (KUCC1391), PGPR-F (KUCC1392), PGPR-G (KUCC1393), PGPR-H (KUCC1394), PGPR-I (KUCC1395), PGPR-J (KUCC1396), PGPR-K (KUCC1397), PGPR-L (KUCC1398), PGPR-M (KUCC1399), PGPR-N (KUCC1400) (Table 1).

Species differentiation and biochemical tests for fluorescent *pseudomonas*: Among the 14 strains of *Pseudomonas* all the strains were differentiated as *Pseudomonas aeruginosa* all the test bacteria grew on nutrient agar supplemented with 5% sucrose (w/v). They showed negative results of exhibiting convex, mucoid colonies after incubation of 2-3 days 25°C which is not indicative of levan formation (Krieg and Holt, 1984). All strains were grown at 41°C which showed positive results for the identification of *P. aeruginosa*. All the test PGPR cultures showed positive results for starch hydrolysis; they formed clear zones around the bacterial colony when flooded with lugol's iodine; these isolates were taken as positive for starch hydrolysis (Table 2).

Table 1. Table showing source of PGPR-strain, locality and species of *Pseudomonas.*

S. No.	Culture	Plant source	Species	Locality
1.	PGPR-A	*Aerva javanica* (Leaves)	*Pseudomonas aeruginosa*	Karachi University (KU)
2.	PGPR-B	*Rhyncosia minima* (Leaves)	*P. aeruginosa*	K U
3.	PGPR-C	*Cressa cretica (*Leaves*)*	*P. aeruginosa*	K U
4.	PGPR-D	*Datura alba* (Leaves)	*P. aeruginosa*	K U
5.	PGPR-E	*Euphorbia hirta* (Leaves)	*P. aeruginosa*	K U
6.	PGPR-F	*Enicostemma verticillatum* (Leaves)	*P. aeruginosa*	K U
7.	PGPR-G	*Phragmites australis* (Leaves)	*P. aeruginosa*	K U
8.	PGPR-H	*Trichodesma indicum* (Leaves)	*P. aeruginosa*	K U
9.	PGPR-I	*Pulchea indica* (Leaves)	*P. aeruginosa*	K U
10.	PGPR-J	*Senna holosercea* (Leaves)	*P. aeruginosa*	K U
11.	PGPR-K	*Pseuda nudiflora* (Roots)	*P. aeruginosa*	K U
12.	PGPR-L	*Aerva javanica* (Roots)	*P. aeruginosa*	K U
13.	PGPR-M	*Pulchea indica* (Roots)	*P. aeruginosa*	K U
14.	PGPR-N	*Pseuda nudiflora* (Leaves)	*P. aeruginosa*	K U

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

Bacterial strains	Growth at 41°C	Levan formation test	Starch hydrolysis
PGPR-A	+	-	+
PGPR-B	+	-	+
PGPR-C	+	-	+
PGPR-D	+	-	+
PGPR-E	+	-	+
PGPR-F	+	-	+
PGPR-G	+	-	+
PGPR-H	+	-	+
PGPR-I	+	-	+
PGPR-J	+	-	+
PGPR-K	+	-	+
PGPR-L	+	-	+
PGPR-M	+	-	+
PGPR-N	+	-	+

Dual culture plate method: The effect of 14 isolates of fluorescent *pseudomonas* against the four root rotting fungi mentioned earlier were examined in a dual culture plate method. PGPR-C, PGPR-B, PGPR-E, PGPR-I, PGPR-k, PGPR-M, PGPR-A and PGPR-D showed maximum inhibition of *Fusarium oxysporum* by producing zones. The best results against *Fusarium solani* were shown by PGPR-N, PGPR-K, PGPR-J, PGPR-E and PGPR-M. While maximum suppression of *Macrophoina phaseolina* was shown by PGPR-D, PGPR-I, PGPR-C, PGPR-K, PGPR-M and PGPR-N as producing zones of inhibition in between 20mm to 8 mm. PGPR-A, PGPR-H, PGPR-D, PGPR-K, showed best results against *Rhizoctonia solani* by inhibiting its growth and making zones of 13 mm. while PGPR-E, PGPR-I and PGPR-N showed lysis of fungal mycelium (Table 3).

Antibacterial activity: Culture filtrates of fourteen plant growth escalating bacteria exhibited noteworthy activity against common lab bacteria. Approximately an inhibition zone of 10 to 30 mm was produced by bacteria (Table 4).

Effect of endophytic fluorescent *Pseudomonas* as soil drench method: This experiment was performed to observe the growth of mung bean plant i.e., Shoot/root length, fresh weight of shoot and root, infection percentage of root-infecting fungi and to determine amount of chlorophyll, carotenoid, protein and carbohydrates after 45 days of PGPR inoculation.

PGPR-J, PGPR-B and PGPR-H, PGPR-I, PGPR-M, showed maximum root length and maximum root weight was shown by PGPR-J, PGPRI, PGPR-B, PGPR-N, PGPR-H, PGPR-I, PGPR-K, PGPR-G. The greater length of shoot was found in plants of strains PGPR-J, PGPR-k, PGPR-I, PGPR-H, and PGPR-I. Whereas maximum shoot weight was observed in plants of strains PGPR-J, PGPR-H and PGPR-B. Maximum number of legumes of mung bean plant was in PGPR-B, PGPR-J, PGPR-D and PGPR-H (Table 5).

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-A	13	3.6	5	13
PGPR-B	19.3	5.3	6.6	***
PGPR-C	28.3	5	11.3	***
PGPR-D	13	5.6	18.6	5.3
PGPR-E	19	7	3.3	**
PGPR-F	11	2.6	9	3**
PGPR-G	12.3	3.3	6.3	*
PGPR-H	11	5.3	8.6	5.6
PGPR-I	16	4.3	8	*
PGPR-J	9	7.3	5.3	2**
PGPR-K	15.3	8	11	4.6
PGPR-L	10	2.3	12	**
PGPR-M	15	6.6	10	*
PGPR-N	6	9.6	9.6	2.5**

*- Test fungus over grew on the bacterium
**- Colonies met each other and fungal mycelium lysed
***- Growth stopped at streak

The root rot fungi were reduced by the following strains. The infection of *Fusarium oxysporum* was controlled greatly by PGPR-C (0%), PGPR-B (2.08%), PGPR-D (2.08%), and PGPR-A (6.25%). PGPR-I and PGPR-I showed great control of *F. solani*. The infection of *Macrophomina phaseolina* was reduced by PGPR-D (2.08%), PGPR-J (4.17 %), and PGPR-E (8.33%). The infection of *Rhizoctonia solani* was greatly reduced by PGPR-J (0%), PGPR-K (0%) (Table 6).

Chlorophyll and carotenoid: Substantial changes were observed in the amount of chlorophyll and carotenoid in mung bean plants that were provided with various isolates of *Pseudomonas* as compared to control. The larger amount of chlorophyll-a was present in PGPR-K, PGPR-M, PGPR-I, PGPR-D, and PGPR-H which was 0.108 mg/g, 0.105 mg/g, 0.103 mg/g, 0.099 mg/g, and 0.098 mg/g, respectively. The amount of chlorophyll-b was greater in PGPR-N 0.046 mg/g, PGPR-B 0.036 mg/g, PGPR-K 0.034 mg/g, PGPR-A 0.024 mg/g, and PGPR-J 0.021 mg/g, the amount of carotenoid was maximum in PGPR-J 0.07 mg/g, PGPR-K 0.05 mg/g, PGPR-D 0.05 mg/g and PGPR-M 0.04 mg/g (Figs. 1, 2 and 3).

Carbohydrates: Substantial increase in the amount of carbohydrates was detected, the large amount of carbohydrates were present in PGPR-J, PGPR-B, PGPR-G, PGPR-I, and PGPR-M which was 349.3 µg/mL, 345.3 µg/mL, 301 µg/mL, 294.6 µg/mL, and 293.3 µg/mL respectively (Fig. 4).

Proteins: Substantial increase in the amount of proteins was detected, the large amount of protein was detected in PGPR-J, PGPR-F, PGPR-I, PGPR-M, and PGPR-E which was 186.6 µg/mL, 186 µg/mL, 185 µg/mL, 183.6 µg/mL and 178 µg/mL respectively (Fig. 5).

Table 4. *In vitro* antibacterial activity of cell free culture filtrates of fluorescent *Pseudomonas*.

Bacterial strain	Treatments	*Bacillus subtilis*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Escherichia coli*
		Zone of inhibition			
PGPR-A	-ve control 20µL/disc	0	0	0	0
	+vecontrol 20µL/disc	19	26.66	17	32.3
	20µL/disc	16.33	14	18.6	16
	40µL/disc	16.66	13	19.3	15
	60µL/disc	18.66	12	21.3	17.3
PGPR-B	+ve control 20µL/disc	17.33	27.66	17.3	34.3
	20µL/disc	14.66	10.66	16.6	18.3
	40µL/disc	12.66	11.66	16.6	18.3
	60µL/disc	14	12	17	19.3
PGPR-C	+ve control 20µL/disc	19.33	25	17	32.6
	20µL/disc	16	14.33	14.3	18.6
	40µL/disc	19.33	14	17.3	12.3
	60µL/disc	18.66	13.66	15	13.6
PGPR-D	+ve control 20µL/disc	17.33	23.33	16.6	31.3
	20µL/disc	14.66	8	12.6	9
	40µL/disc	15.33	8.66	9.6	9.6
	60µL/disc	16	13	13.3	9.3
PGPR-E	+ve control 20µL/disc	14	25.66	20	32.3
	20µL/disc	14.33	15.66	17	13.6
	40µL/disc	14.66	15	17	11.3
	60µL/disc	13	16	17.6	13
PGPR-F	+ve control 20µL/disc	19.66	23.66	20	33.3
	20µL/disc	18.66	14	17.6	12.3
	40µL/disc	20.33	16.66	19.3	12.6
	60µL/disc	17	16.66	23.3	14
PGPR-G	+ve control 20µL/disc	17.66	26	18.3	33.6
	20µL/disc	19	20	13.6	11.6
	40µL/disc	18.66	18	16	10
	60µL/disc	16.33	17.66	19	9
PGPR-H	+ve control 20µL/disc	17	25.33	16.66	27.6
	20µL/disc	15	16.66	17	14.6
	40µL/disc	14.33	14.66	16.6	14.6
	60µL/disc	15.33	15.33	16.3	7.6
PGPR-I	+ve control 20µL/disc	15.3	25	15.66	34.6
	20µL/disc	13	9.66	15.3	16.3
	40µL/disc	12	8.66	14.3	17.6
	60µL/disc	11.33	11	13.6	16.3
PGPR-J	+ve control 20µL/disc	17.66	18.66	15	32
	20µL/disc	15	10.33	15.3	16.3
	40µL/disc	20.66	12	12.6	19
	60µL/disc	17.66	13.66	16	19.3
PGPR-K	+ve control 20µL/disc	14.33	24.33	17	32.3
	20µL/disc	13.33	16.66	14	16.3
	40µL/disc	14	16.33	16.3	18.6
	60µL/disc	12	19.33	19.2	18
PGPR-L	+ve control 20µL/disc	18.66	22.66	14.6	28.6
	20µL/disc	13	17	13.6	21.6
	40µL/disc	14	17.33	14.3	20.6
	60µL/disc	15.66	15.66	14.6	8.3
PGPR-M	+ve control 20µL/disc	16	29	13.6	31.6
	20µL/disc	15.33	10.33	14	15
	40µL/disc	13	15.33	15	18.6
	60µL/disc	14	15	17	18.3
PGPR-N	+ve control 20µL/disc	14.66	27.66	17.66	33.3
	20µL/disc	14.33	20	15.6	14
	40µL/disc	16.33	19	15.3	16
	60µL/disc	12.66	17.33	15.3	13.3

Negative control= Sterilized distilled water Positive control= Penicillin for gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) Streptomycin for gram negative bacteria (*Salmonella typhimurium*, *Escherichia coli*)

Table 5. Effect of endophytic fluorescent *Pseudomonas* and Topspin on the growth of mung bean plant.

Treatment	Root length	Root weight	Shoot length	Shoot weight	No. of legumes	Weight of legumes
Control	$16.8^{a} \pm 7.1$	$1.0^{a} \pm 0.5$	$16.3^{a} \pm 2.8$	$2.9^{a} \pm 1.7$	$0.5^{a} \pm 0.512$	0.5
Topsin	$19.7^{ab} \pm 4.5$	$1.1^{bc} \pm 0.4$	$17.8^{bc} \pm 5.6$	$3.1^{ab} \pm 1.1$	$1.5^{bc} \pm 0.894$	0.5
PGPR-A	$20.0^{ab} \pm 4.9$	$1.2^{abc} \pm 0.4$	$20.7^{cdef} \pm 3.9$	$4.4^{bcde} \pm 2.3$	$2.0^{bc} \pm 1.38$	1.0
PGPR-B	$25.5^{c} \pm 5.7$	$1.8^{d} \pm 1.2$	$23.0^{efg} \pm 3.4$	$5.2^{def} \pm 1.9$	$2.3^{c} \pm 1.30$	1.5
PGPR-C	$22.1^{bc} \pm 5.4$	$1.5^{bcd} \pm 0.8$	$19.1^{bc} \pm 3.1$	$3.7^{abc} \pm 1.5$	$1.6^{bc} \pm 1.20$	0.6
PGPR-D	$21.9^{bc} \pm 5.1$	$1.4^{abcd} \pm 0.5$	$19.3^{bcd} \pm 2.5$	$3.9^{abcd} \pm 1.3$	$2.1^{bc} \pm 0.88$	0.6
PGPR-E	$20.7^{abc} \pm 3.0$	$1.3^{abcd} \pm 0.4$	$20.4^{cde} \pm 2.7$	$4.2^{bcde} \pm 1.9$	$1.9^{bc} \pm 1.28$	1.0
PGPR-F	$19.9^{ab} \pm 3.3$	$1.5^{abcd} \pm 0.3$	$20.6^{cdef} \pm 2.8$	$3.8^{abc} \pm 1.1$	$1.8^{bc} \pm 1.10$	0.6
PGPR-G	$22.8^{bc} \pm 7.0$	$1.7^{cd} \pm 0.8$	$21.8^{def} \pm 3.2$	$4.6^{cde} \pm 1.6$	$1.5^{bc} \pm 1.26$	0.6
PGPR-H	$25.1^{c} \pm 9.0$	$1.8^{d} \pm 0.7$	$23.3^{fg} \pm 2.3$	$5.4^{ef} \pm 1.2$	$2.1^{bc} \pm 0.95$	0.9
PGPR-I	$23.5^{bc} \pm 4.8$	$1.7^{cd} \pm 0.5$	$23.0^{efg} \pm 3.2$	$4.5^{cde} \pm 1.2$	$1.5^{bc} \pm 1.54$	0.5
PGPR-J	$25.5^{c} \pm 6.2$	$1.9^{d} \pm 0.5$	$25.6^{g} \pm 2.0$	$6.1^{f} \pm 1.3$	$2.3^{c} \pm 1.07$	1.7
PGPR-K	$22.0^{bc} \pm 3.9$	$1.7^{cd} \pm 0.5$	$23.4^{fg} \pm 4.0$	$4.6^{cd} \pm e1.82$	$1.5^{bc} \pm 1.03$	0.7
PGPR-L	$24.0^{bc} \pm 6.4$	$1.8^{d} \pm 0.6$	$23.3^{fg} \pm 3.6$	$4.3^{bcde} \pm 1.64$	$1.1^{ab} \pm 1.10$	0.3
PGPR-M	$23.9^{bc} \pm 6.2$	$1.6^{bcd} \pm 0.7$	$20.9^{cdef} \pm 3.4$	$4.1^{abcde} \pm 2.2$	$1.6^{bc} \pm 1.44$	0.7
PGPR-N	$23.3^{bc} \pm 5.0$	$1.8^{d} \pm 0.3$	$23.0^{efg} \pm 2.7$	$4.9^{cdef} \pm 1.1$	$1.8^{bc} \pm 1.27$	0.7

Statistical significance determined by analysis of variance no. followed by the same letter in each column as compared to control are not significantly different (p<0.05) according to Duncan's multiple range test, ± Standard deviation

Table 6. Effect of endophytic fluorescent *Pseudomonas* and Topsin as soil drench on the infection of *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* of mung beans.

Treatment	*Fusarium solani*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Macrophomina phaseolina*
Negative control	$45.83^{b} \pm 6.7$	$41.67^{c} \pm 10.3$	$56.25^{d} \pm 9.2$	$77.08^{d} \pm 9.4$
Positive control	$35.42^{ab} \pm 4.8$	$22.92^{b} \pm 6.4$	$25^{c} \pm 6.1$	$25^{bc} \pm 6.1$
PGPR-A	$25^{ab} \pm 6.8$	$6.25^{a} \pm 4.4$	$6.25^{ab} \pm 4.4$	$10.42^{ab} \pm 4.8$
PGPR-B	$22.92^{ab} \pm 7.1$	$2.08^{a} \pm 2.0$	$14.58^{abc} \pm 5.7$	$8.33^{ab} \pm 3.5$
PGPR-C	$33.33^{ab} \pm 7.1$	$0^{a} \pm 0.0$	$4.17^{ab} \pm 2.8$	$10.42^{ab} \pm 4.8$
PGPR-D	$35.42^{ab} \pm 8.9$	$2.08^{a} \pm 2.0$	$6.25^{ab} \pm 3.2$	$2.08^{a} \pm 2.0$
PGPR-E	$27.08^{ab} \pm 8.4$	$8.33^{a} \pm 4.7$	$2.08^{ab} \pm 2.0$	$8.33^{ab} \pm 3.5$
PGPR-F	$20.83^{a} \pm 5.1$	$4.17^{a} \pm 4.1$	$12.5^{abc} \pm 4.8$	$18.75^{abc} \pm 6.9$
PGPR-G	$27.08^{ab} \pm 8.4$	$4.17^{a} \pm 2.8$	$16.67^{bc} \pm 5.6$	$8.33^{ab} \pm 3.5$
PGPR-H	$31.25^{ab} \pm 8.2$	$8.33^{a} \pm 3.5$	$4.17^{ab} \pm 2.8$	$20.83^{abc} \pm 8.6$
PGPR-I	$16.67^{a} \pm 3.5$	$14.58^{ab} \pm 5.7$	$14.58^{abc} \pm 3.7$	$16.67^{abc} \pm 5.6$
PGPR-J	$37.5^{ab} \pm 8.4$	$6.25^{a} \pm 3.2$	$0^{a} \pm 0.0$	$4.17^{a} \pm 2.8$
PGPR-K	$22.92^{ab} \pm 6.4$	$4.17^{a} \pm 2.8$	$0^{a} \pm 0.0$	$27.08^{bc} \pm 6.4$
PGPR-L	$14.58^{a} \pm 5.7$	$6.25^{a} \pm 3.2$	$16.67^{bc} \pm 6.4$	$33.33^{c} \pm 8.3$
PGPR-M	$20.83^{a} \pm 6.7$	$12.5^{ab} \pm 4.8$	$12.5^{abc} \pm 4.8$	$20.83^{abc} \pm 6.0$
PGPR-N	$25^{ab} \pm 8.1$	$6.25^{a} \pm 3.2$	$8.33^{ab} \pm 4.7$	$10.42^{ab} \pm 5.7$

Statistical significance determined by analysis of variance no. followed by the same letter in each column as compared to control are not significantly different (p<0.05) accorging to Duncan's multiple range test, ± Standard deviation

The Bio-Revolution of 21st century, which encompasses the use of microorganisms, is environmentally friendly and escalates global food production with obscure environmental hazards. The advances in introduction of eco-friendly PGPRs in food production systems is favorable approach to deescalate the usage of chemically synthesized fertilizers. PGPRs protect natural environments along with biological resources by playing a noteworthy role in integrated pest management systems. According to Beattie (2007), biological control managers are defined as agents which deescalate the prevalence of plant diseases, while those that display antagonistic activity against pathogens are termed as antagonists.

Makisimov *et al.,* (2011) and Neeraja *et al.,* (2010) stated that the enzymes namely, chitinases, glucanases, proteases, and lipases are manufactured by antagonistic bacteria which are capable of lysing cells of fungal pathogens, and pathogenic fungal cells. There exists the competition between antagonistic (capable of producing siderophores and antibiotics) and pathogenic organisms in soil for nutrients and suitable colonization of roots (Kamilova *et al.,* 2005). In recent research 14 strains of endophytic fluorescent *Pseudomonas* were extricated from the roorts and shoots of wild plants i.e., *Aerva javanica, Cressa cretica, Datura alba, Euphorbia hirta*, *Enicostemma verticillatum*, *Phragmites australis, Pulchea indica, Rhyncosia minima, Suaeda nudiflora, Senna holosericea, Trichodesma idicum* and the effect of these strains was observed *In vitro* and *In vivo* against root-infecting fungi, pathogenic bacteria and, along with this amount of chlorophyll, carotenoid, protein, and carbohydrates was estimated. These strains were highly effective against soil borne pathogens and remarkably increased in the amount of chlorophyll, protein, and carbohydrates. In this study, tests for differentiation among the species and biochemical tests for characterization of fluorescent *Pseudomonas* bacteria were carried out. All 14 isolates were found to be *Pseudomonas aeruginosa* which showed positive result for growth at 41°C. All isolates were positive to starch hydrolysis test.

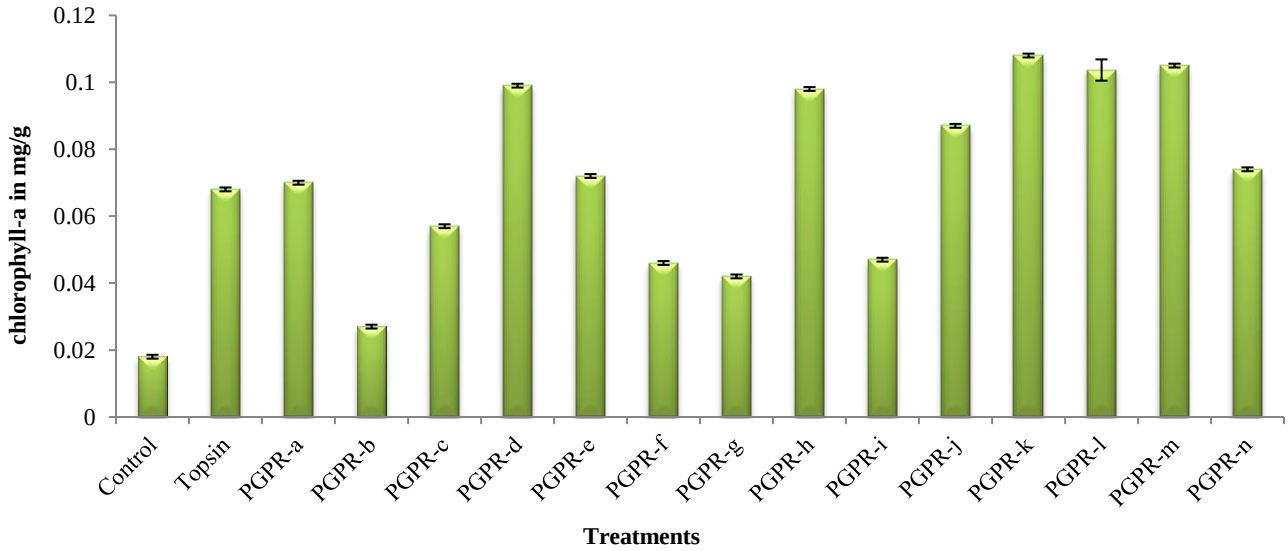

Fig. 1. Effect of endophytic fluorescent *Pseudomonas* and Topsin as soil drench on the amount of chlorophyll-a with standard error.

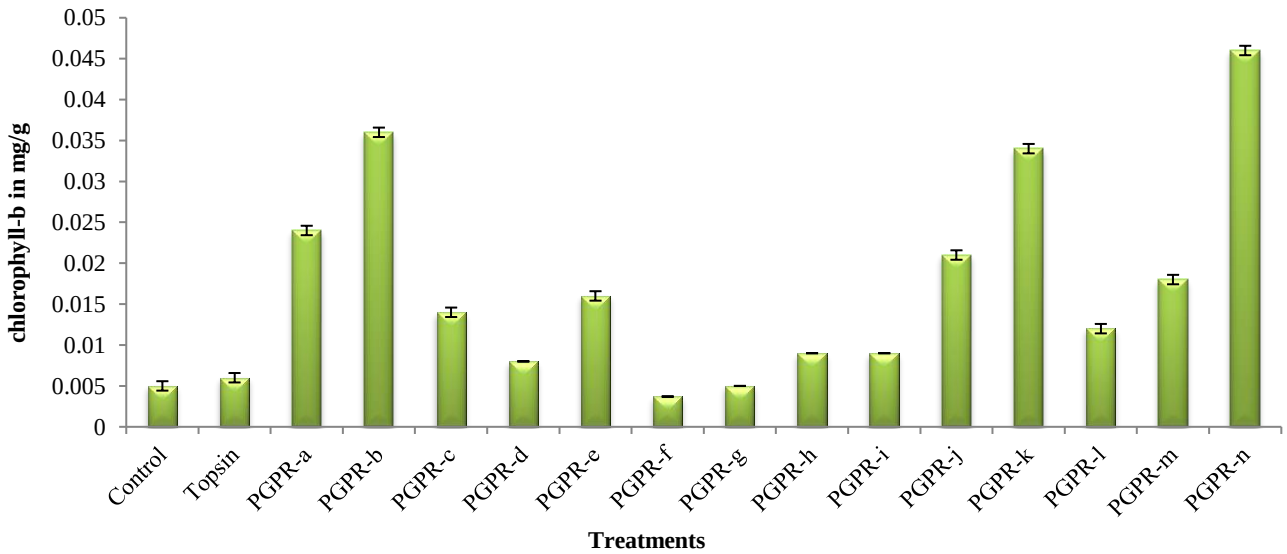

Fig. 2. Effect of endophytic fluorescent *Pseudomonas* and Topsin as soil drench on the amount of chlorophyll-b with standard error.

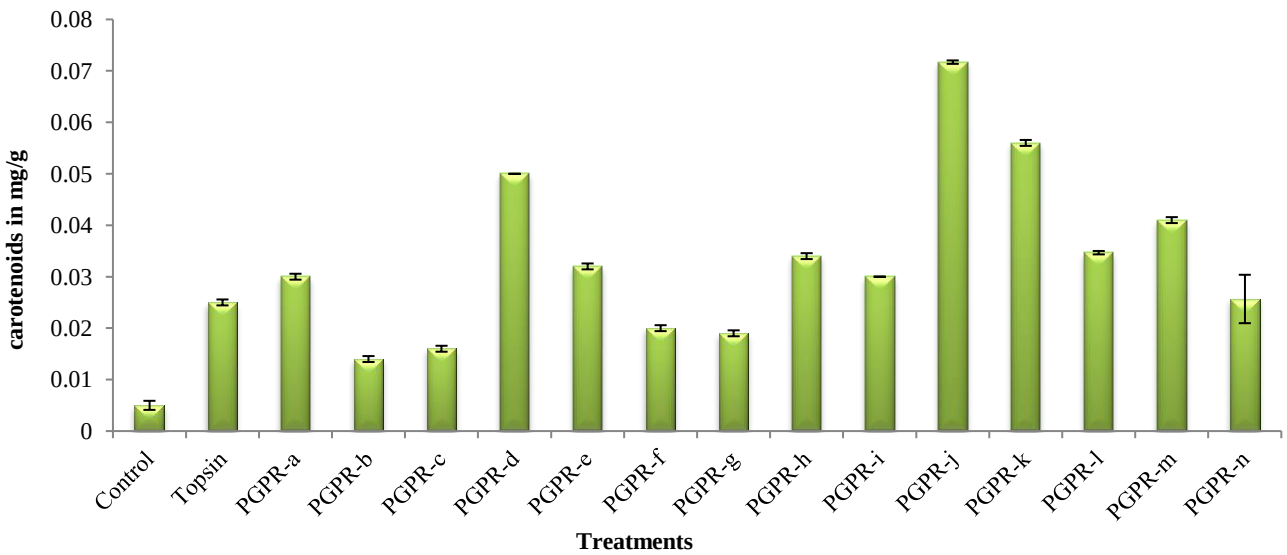

Fig. 3. Effect of endophytic fluorescent *Pseudomonas* and Topsin as soil drench on the amount of carotenoid with standard error.

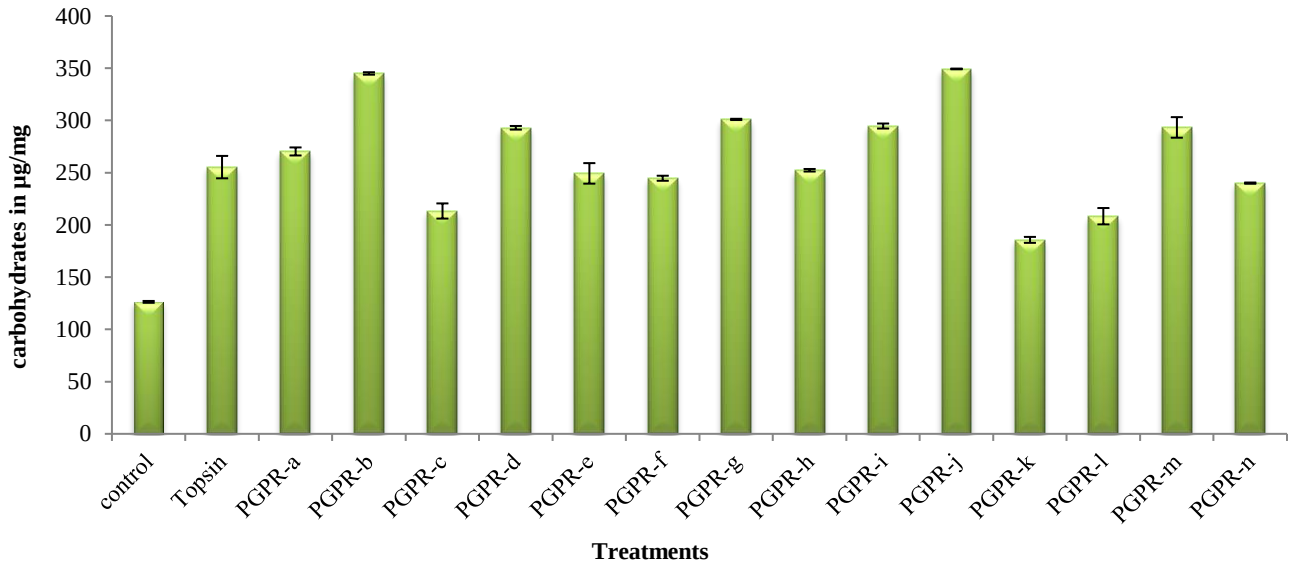

Fig. 4. Effect of endophytic fluorescent *Pseudomonas* and Topsin as soil drench on the amount of carbohydrates with standard error.

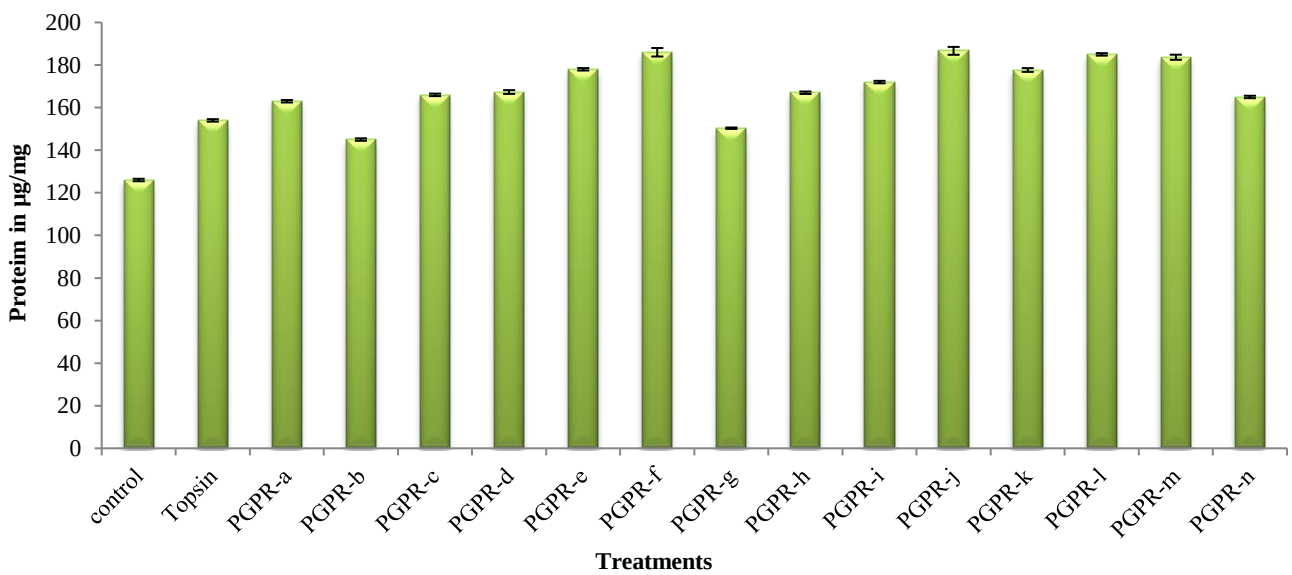

Fig. 5. Effect of endophytic fluorescent *Pseudomonas* and Topsin as soil drench on the amount of protein with standard error.

In dual culture plate method, the effect of 14 different strains of fluorescent *Pseudomonas* against the four root-rot causing fungi was examined *In vitro* and bacterial strains showed inhibitory activity against them. The sec. metabolites production such as phenazines, acetyl phloroglucinols and cyanides gives the antagonistic activity to fluorescent *Pseudomonas* in contrast to plant damaging microorganisms (Jan *et al*., 2011). Bonneau *et al*., (2020) said that the production of siderophores by beneficial bacteria reduces the availability of Iron to plant pathogens. Furthermore, fluorescent *Pseudomonas*' aggressive root colonization is described to play a key part in rhizosphere capabilities and associated biological-control activities (Hass & Keel, 2003). Culture filtrates of fourteen endophytic *Pseudomonas aeruginosa* were observed, all demonstrated very strong antibacterial action counter to all of the four test bacteria by developing inhibition zones. The activity of cell-free filtrate of PGPR cultures against the bacteria was reported analogous with the commercial antibiotic streptomycin and penicillin at 20 µL/disc. According to several findings, *Pseudomonas* is efficient against bacterial pathogens in addition to its activity against root rotting fungi (Manav & Thind, 2002; Tsai *et al*., 2004). Pots treated with fluorescent *Pseudomonas* exhibit a noteworthy surge in root and shoot length and weight over the untreated control and Topsin. The growth and nutrition of plants could be enhanced by Plant growth-promoting bacteria, therefore growing plant resistance against pathogens (Compant *et al*., 2005 & Liu *et al*., 2012). Shafique *et al*., (2015a), Shafique *et al*., (2015b) and Rehman *et al*., (2016) reported that the *P. aeruginosa* significantly increases the systemic resistance in okra and cotton. *Pseudomonades* are well-known for the production of indol acetic acid and gibberellic acid, they promote the growth of plants and they have capability of phosphate solubilization (Megha, 2006). These properties enable *Pseudomonas* to be reliable bio-control mediator in controlling soil borne pathogens (Haas & Defago, 2005).

The necessary constituent of plant pigments which play vital role in the process of photosynthesis is chlorophyll. Photosynthesis cannot take place in the absence of a suitable amount of this pigment. According to Alberts *et al*., (2002) chlorophyll plays pivotal role in ATP production and preservation of important plant elements. It is used to assess plant defensive capabilities. The amount of chlorophylls is the marker of metabolic activity and photosynthetic activity (Wright & Jones, 2006; Hartmann *et al*., 2009). The amount of chlorophyll and carotenoid was determined which showed an increase in their amount. In this study the larger amount of chlorophyll-a was present in PGPR-k, PGPR-M, PGPR-I, PGPR-D, and PGPR-H. Bashan *et al*., (1990) reported the escalated chlorophyll amount in plant leaves consequently of bacteria, integrated application could be owing to the increased buildup of photosynthesis and Plant nutrition. The application of PGPR efficiently amplified the amount of chlorophyll in strawberries (Karlidag *et al*., 2013). The amount of carotenoid seemed to be enhanced by *Pseudomonas* strains. The use of several PGPR strains demonstrated that a rise in carotenoid was detected following integrated application of *Azospirillum* and *Pseudomonas* in both normal and stress settings (Saghafi *et al*., 2013). Kang *et al*., (2014) showed increased amount of chlorophyll in PGPR-treated *Cucumis sativus* plants under salinity and drought stress. Improved amounts of carbohydrates were detected, the large amount of carbohydrates were also existent in PGPR strains. Protein is among the reserve food materials used by plants for the development of the seedling. Significant rise in the amount of proteins was observed, the large amount was present in PGPR-J, PGPR-F, PGPR-I, PGPR-M, and PGPR-E. Dhanaya & Adeline (2014) reported the elevation of growth, biochemical parameters like proteins, and carbohydrates by the application of *Pseudomonas, Rhizobium,* and P solubilizing bacteria in *V. radiata.* Adesemoye & Kloepper (2009) reported that the PGPR interactions with plants results in the increased amount of protein. According to Basu *et al*., (2008) the applicatoin of plant growth promoting rhizobacteria gave rise to the content of protein in crop plants.

The preliminary use of fluorescent *Pseudomonas* toughens host cell wall structure that results in restriction on the invasion of of pathogen of tissues of plant (Benhamou *et al*., 2000; Chen *et al*., 2000; Conrath *et al*., 2002; Dwivedi & Johri, 2003). In this study, fluorescent *Pseudomonas* play the similar role by preventing root rotting pathogens from entering the host plant inhabiting the soil. The remarkable results revealed that *Fusarium oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* were controlled maximum as compared to the *Fusarium solani.* However, an efficient decline in the severity of disease was also observed in other root-infecting fungi. PGPR rhizobia has also been reported to reduce prevalence of pathogenic root-rot causing fungi nematodes causing root-knots on leguminous and non-leguminous crops (Ehtesham-ul-Haque & Ghaffar, 1993). Herein this research, some of the *Pseudomonas aeruginosa* strains showed substantial activity in controlling root rotting fungi, and pathogenic bacteria. There are several reports that reveal the mutual interaction of PGPR with host plants (Pandey *et al*., 2005).

Conclusions

The biocontrol based on PGPR shows promising results, and it mitigates the usage of synthetic fertilizers for the protection of crop plants. Broad application of PGPR revealed their efficiency and acceptability, not only by the regulatory agencies but producers of crops also. Production of crops with high yield and nutritional value in the green houses is an exalted approach for PGPR utilization. On the basis of successful performance of PGPR in green houses, the benefit of controlled conditions of environment could be a more convenient place where initial research, management of diseases, and escalation of the quality of crop plants are carried out.

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