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ROLE OF MYCORRHIZOSPHERIC FLUORESCENT *PSEUDOMONAS* IN SUPPRESSING THE ROOT ROT DISEASE, ENHANCEMENT OF VESICULAR ARBUSCULAR MYCORRHIZAL (VAM) POPULATION AND PHOSPHORUS UPTAKE IN SUNFLOWER

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Abstract

The vesicular arbuscular mycorrhizal (VAM) symbiosis is known to improve nutrient uptake by plants particularly phosphorus, and suppress soilborne plant pathogens. However, mycorrhizospheric bacteria also affect VAM fungi and their host plant. A total of 87 isolates of fluorescent *Pseudomonas* (MRFP) were isolated from the mycorrhizosphere of 15 plant species in this study. These isolates were initially identified on the basis of biochemical tests. Molecular biology tools (16S rDNA gene sequencing) were used to confirm the identification of promising isolates. These isolates were evaluated against root rot pathogens, *Macrophomina phaseolina, Fusarium solani, F.oxysporum* and *Rhizoctonia solani* and *Meloidogyne javanica,* a root knot nematode *in vitro,* suppressed growth of most of the fungi and demonstrated strong nematicidal activity. Performance of promising isolates of MRFP were evaluated on sunflower in pots and field plot experiments showed significant suppressive effect on root rot pathogens resulting in the production of taller plants having greater shoot weight and flower weight as compared to control plants. Application of MRFP both in pots and field plot experiments significantly increased VAM population around roots and generally improved phosphorus uptake by plants. MRFP was found very effective in ameliorating activity of native VAM. It seems that MRFP plays some role in the stimulation and proliferation of VAM fungi in plant-fungus interaction.

Key words: Fluorescent *Pseudomonas,* Root diseases, VAM population, Phosphorus uptake.

Introduction

Vesicular arbuscular mycorrhizae (VAM) are known to form symbiotic relations with about 80% of plant species. VAM helps plants in nutrient uptake, especially phosphorus, improve tolerance to stresses and mobilization of minor elements (Vander Heijden *et al.,* 2015) and protect plants from abiotic and biotic stresses (Agnolucci *et al.,* 2019). They attenuate the severity of fungal diseases (Bokhari *et al*., 2014), root knot nematode's infestation (Akhtar & Siddiqui, 2007) and ameliorate production of beneficial plant metabolites which ultimately play a role in the production of high quality food (Avio *et al*., 2018). However, activity of VAM fungi may be affected by the associated bacteria (Johansson *et al.,* 2004). Mycorrhizospheric bacteria are reported for the promotion of mycorrhizal activity (Agnolucci *et al*., 2015) and protect plants from soilborne pathogens (Bokhari *et al*., 2013). Bacteria associated with VAM may solubilize phosphorus, besides inhibiting the pathogenic fungi and producing indole acetic acid (IAA) (Cruz & Ishii, 2011).

Among various rhizosphere and mycorrhizosphere bacteria fluorescent *Pseudomonas* has been isolated from plant roots and also from inside plant tissues (Afzal *et al*., 2013; Moin *et al*., 2020). They are also known to suppress plant pathogens attacking plant roots by directly inhibiting them or by inducing systemic resistance (Korejo *et al*., 2017; 2019). Siddiqui & Mahmood (2001) have reported a better control of *Meloidogyne javanica* with enhancement in the growth of chickpea by mixed application of *Glomus mosseae* and *Pseudomonas fluorescens* than either used alone. Colonization of mycorrhiza was increased when applied with *Pseudomonas putida* (Akhtar & Siddiqui, 2007). *Glomus deserticola* caused greater reduction of *Pythium aphanidermatum* in chickpea when used with *P. fluorescens* (Nwaga *et al*., 2007). Fluorescent *Pseudomonas* has also been reported to suppress root rotting fungi and improve mycorrhizal activity on sunflowers and tomatoes (Ehteshamul-Haque *et al*., 2015). This study describes the influence of MRFP on VAM fungi to suppress the root diseases and also enhance sunflowers growth in pots as well as field plot experiments. This report highlights the role of MRFP on VAM population and phosphorus uptake by the sunflower plants.

Materials and Methods

Isolation and identification of fluorescent *Pseudomonas* from mycorrhizospheric soil: To isolate mycorrhizospheric fluorescent *Pseudomonas*, healthy plants were collected from different agricultural fields of Lower Sindh. Roots along with adhering soil of 5 specimens per crop from each field were collected. Fluorescent *Pseudomonas* was isolated from mycorrhizosphere on Petri dishes containing S-1 medium within 24 hour (Afzal *et al.,* 2013; Gould *et al*., 1985). Bacterial colonies fluoresce under UV light after 3 d growth at 28^{o}C was purified (King *et al*., 1954).

Isolation of VAM spores from soils and their identification: VAM spore in mycorrhizosphere soils was extracted by decanting and wet sieving technique as described by Gerdemann & Nicolson (1963); identified after reference to Schenck & Perez, (1990).

Molecular identification of fluorescent *Pseudomonas*: Bacterial DNA was isolated from pure culture by using Genomic DNA Mini-Preps Kit (Biobasic, Canada) according to instruction. To assess DNA quality, 1% agarose gel was used, while the purity and concentration of DNA was estimated by spectrophotometer (Shimadzu UV-1800), Japan). Primers sets PA-F5'-ACTGACACTGA-GGTGCGAAAGCG-3' and PA-R3'-ACCGTATGCGC TTCTTCACTTG ACC-5' were used to amplify the 16S rDNA region (Noreen *et al*., 2015), using BioRad ABI 2700 thermal cycler (California, USA). For the amplification of targeted region, PCR reaction mixture was comprised of bacterial DNA 50 ng, 1 µL of forward and reverse primer (10 µM), 25 µL of 2x DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA) upto final volume of 50 µL with Nuclease Free water (Korejo *et al*., 2019). To identify bacterial isolates, BLAST analysis was done and their phylogenetic relationship of all isolates was constructed by neighbor joining (NJ) method using MEGA-X software. Sequences were submitted to NCBI Genbank under accession numbers MN850312 for MRFP-201, MN850313 for MRFP-202, MN850314 for MRFP-206 and MN850315 for MRFP-212.

Determination of antifungal activity: Inhibitory effect of mycorrhizospheric *Pseudomonas* on root rotting fungi was evaluated by dual culture plate assay on Czapek'sDox Agar (Ji *et al*., 2014; Siddiqui *et al*., 2001). Test fungus was inoculated on one side of the Petri dishes while test bacterium was streaked on other side and incubated at 28^{o}C upto 7 days and their zones of inhibition were measured.

Bacterial culture filtrates and nematicidal activity: Test bacterium was grown in KB broth at 30^{o}C for 2 days then centrifuged at 3000 rpm for 20 minutes, supernatant was collected and used for nematicidal activity. Supernatant/ cell free culture filtrate (1 mL) of test bacterium was transferred in glass cavity block and aqueous suspension of nematode root knot (*Meloidogyne javanica)* (1 mL of freshly hatched 2nd stage juvenile- 20 juveniles) was added. They kept at 26 ± 5^{o}C while juvenile mortality was recorded after 48 hours (Siddiqui *et al*., 2001).

Effect of MRFP on sunflower in screen house experiment: The garden soil used in this experiment was naturally infested with *Macrophomina phaseolina* (4-8 sclerotia/g soil), *Rhizoctonia solani* (3-7% colonization of sorghum seeds) and *Fusarium* spp., (3000 cfu/g soil) as determined by using techniques of Sheikh & Ghaffar (1975), Wilhelm, (1955) and Nash and Snyder (1962) respectively. In clay pots (1 Kg soil), cell suspension of *Pseudomonas* isolates viz., MRFP-202, MRFP-203, MRFP-206, MRFP-211 and MRFP-212 were applied at 25 mL/ pot. Carbendazim (25 mL/200 ppm) served as positive control against root rotting fungi. In another set, pots were given mixed application of *Pseudomonas* and VAM spore (*Glomus* sp.) extracted from rhizosphere soil of sunflower, at100 spores/pot. In each pot (6 seeds per pot) seeds of sunflower (*Helianthus annuus*) variety HO-1, were sown. Four seedlings in each pot were maintained after germination and randomized in block design. Effect of MRFP on plant growth (shoot & root length and fresh root & shoot weight), incidence of root infecting fungi, VAM population and phosphorus uptake was determined after six weeks. For determining the incidence of each fungus on the root, tap roots were cut into (1 cm) pieces and then surface sterilized with 1% bleach. The small pieces of roots were placed on PDA plates supplemented with antibiotics, streptomycin (0.2 g/ L) and Penicillin (100000 unit/L). Fungi grown from root pieces after 5 days of incubation at 25^{o}C were identified and infection (%) of each fungus was calculated as describe by Noreen *et al*., (2015):

$$\text{Infection \%} = \frac{\text{Total no. of plants infected by a fungus}}{\text{Total number of plants}} \times 100$$

The phosphorus was determined in plants by dry ash method as described by Rayan *et al*., (2001).

Evaluation of MRFP in suppressing root rot disease of sunflower in field plot experiments (2014): Efficacy of fluorescent *Pseudomonas* examined at the field plot (2x2 m) in the Department of Botany in March 2014. A natural population of (2-5 spores/g) of VAM was found in soil, besides infestation of root infecting fungi *M. phaseolina* (5-13 sclerotia/g), *R.solani* (3-14 % colonization of sorghum seeds) and *Fusarium* spp., (3100cfu/g). Seeds (50) of sunflower were sown (in two meter row) and cell suspension (10^{8}cfu/mL) of fluorescent *Pseudomonas* MRFP-202, MRFP-203, MRFP-206 and MRFP-212 were drenched in each row (200 mL). A commercial fungicide carbendazim 200 mL (200 ppm in water) per 2 meter row was kept as positive control while plants not receiving any treatment considered as control. The experiment was conducted with 4 replicates using complete block design. Observations were recorded at 30 and 60 day of experiment. To confirm the results, the whole experiment was repeated in 2015 in similar condition.

Data analysis: Software (CoStat, CA, USA) was used for analysis of variance (ANOVA) and determination of Least Significant Difference (LSD) at (p<0.05).

Results

Isolation of fluorescent *Pseudomonas* from mycorrhizosphere: From mycorhizopshere of 15 plant species viz., *Abelmoschues esculentus* L., *Amaranthus* sp., *Carica papaya* L., *Capsicum annuum* L., *Cyamopsis tetragonoloba* L., *Momordica charantia* L., *Musa acuminata* Colla., *Luffa aegyptiaca* Mill., *Lycopersicon esculentum* Mill., *Pennisetum americanum* (L.) R.Br., *Sesbania sesban* (L.) Merrill, *Solanum melongena* L., *Triticum aestivum* L., *Vigna radiata* (L.) R. Wilczek and *Zea mays* L. 87 isolates of fluorescent *Pseudomonas* were isolated and identified (Table 1). Species of *Glomus* were found predominant in the mycorrhizosphere of most of the plants, However, *Acaulospora, Entrophospora* and *Gigaspora* were also found associated with plants (Table 1).

Table 1. Fluorescent *Pseudomonas* isolated from mycorrhizosphere of crop plants.

***Pseudomonas* isolates**	**VAM association**	**Plant source**	**Locality**
MRFP-201	*Glomus fasciculatum. Entrophospora*	*Momordica charantia*	M
MRFP-202	*Glomus mosseae, G. intraradices, Gigaspora* sp.	-	-
MRFP-203	*Glomus fasciculatum, G. mosseae, G. geosporum, Entrophospora*	*Solanum melongena*	-
MRFP-204	*Glomus fasciculatum, G mosseae, G. neosporum, Entrophospora*	-	KT
MRFP-205	*Glomus Fasciculatum, G. mosseae, G.aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, G. monosporum, Entrophospora, Gigaspora*	*Lycopersicon esculentum*	M
MRFP-206	*Glomus fasciculatum,G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Gigaspora, G. aurantium, Entrophospora, Gigaspora*	*Capsicum annuum*	KT
MRFP-207	*Glomus fasciculatum, G. mosseae*	*Sesbania sesban*	KT
MRFP-208	*Glomus fasciculatum, G. mosseae, G.geosporum*	*Solanum melongena*	-
MRFP-209	*Glomus fasciculatum, G. geosporum, Entrophospora*	*Zea mays*	-
MRFP-210	*Glomus fasciculatum, G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Gigaspora, Acaulospora* sp.	*Lycopersicon esculentum*	-
MRFP-211	*Glomus fasciculatum, G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Gigaspora, Acaulospora* sp.	-	MG
MRFP-212	*Glomus* sp. *G. fasciculatum, G. geosporum, Entrophospora*	*Zea mays*	-
MRFP-213	*Glomus fasciculatum, G. geosporum, Entrophospora*	-	-
MRFP-214	*G. fasciculatum,G. geosporum, G. frailistratum, Entrophospora*	*Amaranthus* sp.	-
MRFP-215	*G. fasciculatum, G. geosporum, G. frailistratum, Entrophospora*	-	-
MRFP-216	*G. fasciculatum*	*Carica papaya*	-
MRFP-217	*Glomus* sp.	*Pennisetum americanum*	-
MRFP-218	*Glomus fasciculatum, G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Gigaspora margarita, Entrophospora, Gigaspora, Acaulospora* sp.	*Lycopersicon esculentum*	-
MRFP-219	*Glomus fasciculatum, Gmossae, Entrophospora*	*Sesbaniasesban*	-
MRFP-220	*Glomus fasciculatum, G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Acaulosporasp, G. aurantium, Entrophospora, Acaulospora* sp.	*Lycopersicon esculentum*	-
MRFP-221	*Glomus fasciculatum, G. geosporum, G. frailistratum*	*Momordica charantia*	-
MRFP-222	*Glomus fasciculatum, G. geosporum, G. frailistratum*	-	-
MRFP-223	*Glomus fasciculatum, G. mosseae*	*Sesbania sesban*	-
MRFP-224	*Glomus fasciculatum, G. mossae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Gigaspora, Acaulospora* sp.	*Lycopersicon esculentum*	DC
MRFP-225	*Gigaspora* sp., *Acaulospora* sp.	-	KU
MRFP-226	*Gigaspora* sp., *Acaulospora* sp.	*Abelmoschus esculentus*	-
MRFP-227	*Glomus fasciculatum, G. mosseae, G. frailistratum*	*Vigna radiata*	-

Table 1. (Cont'd.).

Pseudomonas isolates	VAM association	Plant source	Locality
MRFP-228	*Glomus fasciculatum, G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Gigaspora* sp.	*Capsicum annuum*	MG
MRFP-229	*Glomus fasciculatum, G. mosseae, G. aggregatum, G. intraradices, G. geosporum, G. frailistratum, G. aurantium, Entrophospora, Gigaspora, Acaulospora* sp.	*Lycopersicon esculentum*	-
MRFP-230	*Glomus fasciculatum*	*Musa acuminate*	M
MRFP-231	*Glomus fasciculatum, G. mosseae, G .frailistratum*	`	-
MRFP-232	*Glomus fasciculatum, G. mosseae, G. geosporum*	*Solanum melongena*	-
MRFP-233	*Glomus fasciculatum, G. mosseae, G. intraradices, G. geosporum, G. frailistratum, Entrophospora, Gigaspora, Acaulospora* sp.	*Lycopersicon esculentum*	MG
MRFP-234	*Glomus mosseae, G. geosporum, G. intraradices, Gigaspora, Entrophospora, Acaulospora* sp	-	KT
MRFP-235	*Glomus fasciculatum, G. mosseae, G. intraradices, Entrophospora, Gigaspora, Acaulospora* sp.	-	DC
MRFP-236	*Glomus mosseae, G. intraradices, G. geosporum, G. frailistratum, Entrophospora* sp., *Gigaspora* sp.	-	M
MRFP-237	*G. mosseae, G. intraradices, G. geosporum, Entrophospora* sp., *Gigaspora* sp., *Acaulospora*	-	TH
MRFP-238	*Glomus fasciculatum, G. mosseae, G. intraradices, G. geosporum, Gigaspora* sp.	-	GH
MRFP-239	*Glomus fasciculatum,G.mosseae, G. intraradices, G. geosporum, Entrophospora* sp., *Gigaspora* sp., *Acaulospora* sp.	-	KU
MRFP-240	*Glomus fasciculatum, Gigaspora* sp.	*Carica papaya*	MG
MRFP-241	*Glomus intraradices, Glomus* sp.	-	KT
MRFP-242	*Glomus fasciculatum, Acaulospora* sp.	-	DC
MRFP-243	*Glomus* sp. *Gigaspora* sp.	*Cyamopsis tetragonoloba*	KT
MRFP-244	*Glomus* sp. *Gigaspora* sp.	-	DC
MRFP-245	*Glomus* sp. *Gigaspora* sp.	-	MG
MRFP-246	*Glomus fasciculatum, G. mosseae, Gigaspora* sp.	*Triticum aestivum*	KT
MRFP-247	*Glomus mosseae, Acaulospora* sp., *Glomus* sp.	-	M
MRFP-248	*Glomus* sp. *Gigaspora*	-	DC
MRFP-249	*Acaulospora* sp., *Glomus fasciculatum, Glomus* sp.	-	GH
MRFP-250	*Glomus* sp., *Acaulospora* sp.	*Luffaaegptiaca*	KT
MRFP-251	*Glomus mosseae, Gigaspora* sp.	-	-
MRFP-252	*Glomus* sp.	-	M
MRFP-253	*Glomus* sp.	-	-
MRFP-254	*Glomus fasciculatum, Glomus* sp.	-	DC
MRFP-255	*Glomus fasciculatum, G. geosporum*	*Zea mays*	KU
MRFP-256	*Glomus fasciculatum, G. geosporum*	-	-

Table 1. (Cont'd.).

***Pseudomonas* isolates**	**VAM association**	**Plant source**	**Locality**
MRFP-257	*Glomus fasciculatum, G. geosporum*	-	KT
MRFP-258	*Glomus fasciculatum, G. geosporum*	-	-
MRFP-259	*Glomus fasciculatum, G. geosporum*	-	MG
MRFP-260	*Glomus fasciculatum, G. geosporum*	-	KU
MRFP-261	*Gigaspora, Acaulospora* sp.	*Abelmoschus esculentus*	-
MRFP-262	*Gigaspora, Acaulospora* sp.	-	KU
MRFP-263	*Gigaspora, Acaulospora* sp.	-	M
MRFP-264	*Gigaspora, Acaulospora* sp.	-	-
MRFP-265	*Glomus fasciculatum, G. mosseae, G. intraradices, G. geosporum, Gigaspora* sp.	*Lycopersicon esculentum*	KU
MRFP-266	*Glomus fasciculatum, G. mosseae, G. intraradices, G. geosporum, Gigaspora* sp.	-	KT
MRFP-267	*Glomus fasciculatum, G. mosseae, G. intraradices, G. geosporum, Gigaspora* sp.	-	-
MRFP-268	*Glomus fasciculatum, G. mosseae, G. intraradices, G. geosporum, Gigaspora* sp.	-	M
MRFP-269	*Glomus fasciculatum, Gigaspora* sp.	*Carica papaya*	KU
MRFP-270	*Glomus fasciculatum, Gigaspora* sp.	-	MG
MRFP-271	*Glomus fasciculatum, Gigaspora* sp.	-	KT
MRFP-295	*Glomus fasciculatum, G. mosseae, G. frailistratum*	*Vigna aadiata*	M
MRFP-303	*Glomus* sp. *Gigaspora* sp.	*Cyamopsis tetragonoloba*	SK
MRFP-304	*Glomus fasciculatum, G. mosseae, G. frailistratum*	*Vigna radiata*	KU
MRFP-305	*Glomus fasciculatum, G. mosseae, Glomus* sp.	-	M
MRFP-306	*Glomus mosseae, Glomus* sp.,	-	-
MRFP-307	*Glomus fasciculatum, G. mosseae*	-	KU
MRFP-308	*Glomus fasciculatum, G. mosseae, Acaulospora* sp.	-	KT
MRFP-309	*Glomus geosporum, G. mosseae*	-	KU
MRFP-310	*Glomus intraradices, G. mosseae, Entrophospora* sp.,	-	DC
MRFP-311	*Glomus fasciculatum, G. mosseae, G. frailistratum*	-	KU
MRFP-312	*Glomus fasciculatum, G. mosseae, Gigaspora* sp., *Acaulospora* sp.	-	-
MRFP-313	*Glomus mosseae, G. frailistratum, Acaulospora* sp.	-	SK
MRFP-314	*Glomus fasciculatum, G. mosseae*	-	SK
MRFP-316	*Glomus fasciculatum, G.intraradices, G. mosseae, Entrophospora* sp.	-	KU
MRFP-317	*Glomus fasciculatum, G. mosseae*	-	M
MRFP-318	*Glomus fasciculatum, G. intraradices, G. mosseae, Entrophospora* sp.	-	KU

KU= Karachi University field, KT= Kathor, M= Malir, MG= Memon Goth, DC= Darsano Channo, TH= Thatta, GH= Gharo, SK= Sakroo

Molecular identification of promising isolates of fluorescent *Pseudomonas*: Biochemical tests, as described in Bergey's manual were used to identify fluorescent *Pseudomonas* tentatively, while molecular biology tools were used to confirm the identification of promising isolates. DNA sequence alignment of the selected fluorescent *Pseudomonas* strains i.e., MRFP-201, MRFP-202, MRFP-206, MRFP-212 indicated that nucleotide sequence of MRFP-206 and MRFP-212 were similar compared to other two strains. The phylogenetic tree of these isolates constructed by neighbor joining methods exhibited that *Pseudomonas* strains MRFP-206 and MRFP-212 showed slight diversity with MRFP-202 than MRFP-201 (Fig. 1).

***In vitro* inhibition of root rotting fungi by MRFP:** Out of 87 isolates of MRFP tested for antifungal activity, 27 isolates were found to inhibit radial growth of all four test root rot fungi *R. solani*, *F.oxysporum*, *F.solani* and *M. phaseolina* by producing zone of inhibition. Some of these isolates also caused lysis of fungal hypae (Table 2).

Nematicidal activity of MRFP: Out of 77 isolates, mycorrhizospheric fluorescent *Pseudomonas* (MRFP) examined for nematicidal activity, 30 of them showed 100 % mortality of juveniles of *M. javanica* within 48 hours, while 41 killed more than 50% within 48 hours (Table 2).

Effect of MRFP on sunflower in screen house experiment: Fluorescent *Pseudomonas* MRFP-206 or *Glomus* sp alone or mixed application showed no infection of *F.solani.* (MRFP-203, MRFP-206, MRFP-211, MRFP-212) and *Glomus* sp., reduced *F.oxysporum* on roots. The application of MRFP and *Glomus* sp., collectively suppressed *R. solani* infection as used separately or VAM applied with fluorescent *Pseudomonas. Pseudomonas* or VAM did not show the effective result in the control of *M. phaseolina* when used alone. However, the strain of MRFP-203 combined with VAM significantly suppressed the infection of *M. phaseolina* (Table 3).

The combined application of MRFP-203, MRFP-211 and MRFP-206 with *Glomus* sp., showed enhanced plant height (Table 2). Phosphorus conc., was found to increase in plants that received culture of fluorescent *Pseudomonas* (MRFP-202, MRFP-203 and MRFP-212) (Table 2). The population of VAM spores around root soil was also found in greater numbers in treated plants than those plants which did not receive any bacterial culture (Table 4).

Effect of MRFP on sunflower in field plot experiments (2014): After 30 days MRFP-203 significantly inhibited the *R. solani* and *M. phaseolina*, whereas MRFP-202 was effective against *M. phaseolina* (Table 5). Application of fluorescent *Pseudomonas* resulted in the improvement of growth of treated plants as evident from the taller plants with greater fresh shoot weight in bacterized treatment than control plants (Table 6). MRFP treated plants also showed a higher number of VAM spores around roots than other treatments (Table 6).

After 60 days, the infection of *R. solani* and *Fusarium* spp., found less in all treatments (Table 5). Application of MRFP-211 and MRFP-206 found effective to suppress the infection of *M. phaseolina* rather than normal control (Table 6). The bacterial treated plants showed effective results with taller plants and increased fresh shoot weight (Table 6). Increased plant height with maximum fresh shoot weight achieved by the application of MRFP-203(Table 6). Plants treated with bacterial culture were found to have an increased number of VAM populations in the roots vicinity than control plants. Where maximum spore population was observed around the roots treated with MRFP-202 (Table 6).

Effect of MRFP on sunflower in field plot experiments (2015): *Rhizoctonia solani* and *M. phaseolina* on sunflower roots were found greater than last year, but most of the MRFP isolates were found effective in suppressing their infection compared to untreated control plants. Their efficacy was compared with carbendazim, a commercial fungicide. Infection of *F. oxysporum* and *F. solani* was generally found less in most of the treatment in both 30 and 60 day observations (Table 7). MRFP treated plants showed significantly better plant growth like plant height and fresh shoot weight at 60 days rather than untreated control plants (Table 8). Population of VAM around roots and phosphorus uptake by plants were found greater in plants that received MRFP than untreated control (Table 8).

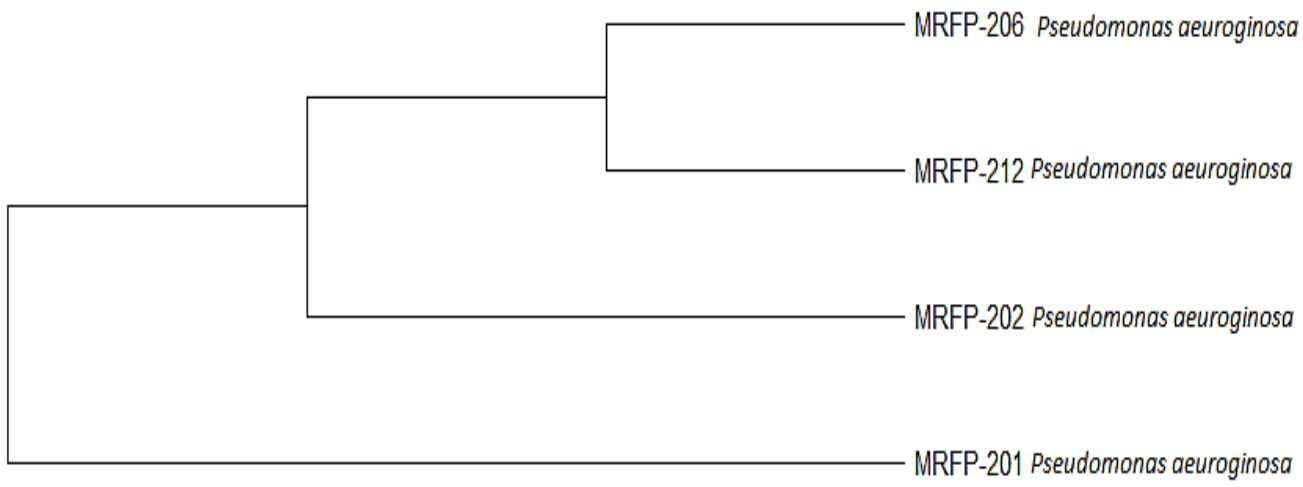

Fig. 1. Phylogenetic tree of *Pseudomonas* strains showing relatedness among them.

Table 2. *In vitro* growth inhibition of *Fusarium solani, F.oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* and nematicidal activity against 2nd stage juveniles of *Meloidogyne javanica* by the mycorrhizospheric fluorescent fluorescent *Pseudomonas* (MRFP) isolated from mycorrhizosphere.

Pseudomonas isolates	*F. solani*	*F. oxysporum*	*R. solani*	*M. phaseolina*	Juvenile mortality
	Zone of inhibition (mm)			% After 48 hrs.	
Control	--	--	--	--	00
MRFP-201	31	30	4.0	33	100
MRFP-202	38	13	30	30	100
MRFP-203	28	28	10	33	100
MRFP-204	23	24	16	20	100
MRFP-205	34	34	28	5	100
MRFP-206	21	34	28	5	100
MRFP-207	28	28	4	33	100
MRFP208	31	27	10	0	90
MRFP-209	25	28	3	35	100
MRFP-210	32	31	0	0	100
MRFP-211	26	27	2	7	100
MRFP-212	28	31	4	5	100
MRFP-213	31	18	0	16	100
MRFP-214	39	16	0	4.5	100
MRFP-215	38	22	0	32	100
MRFP-216	34	14	2	34	100
MRFP-217	33	21	0	32	100
MRFP-218	33	32	0	10	100
MRFP-219	35	36	4	0	88
MRFP-220	39	36	0	0	100
MRFP-221	39	31	0	3	100
MRFP-222	37	31	0	0	100
MRFP-223	21	22	0	0	85
MRFP-224	25	22	21	2	100
MRFP-225	17	24	0	14	100
MRFP-226	25	18	7	19	100
MRFP-227	22	19	3	0	95
MRFP-228	28	23	10	25	100
MRFP-229	18	21	0	0	100
MRFP-230	17	21	0	0	100
MRFP-231	35	20	0	27	100
MRFP-232	24	26	0	5	100
MRFP-233	27*	26.6	15	18.6	88
MRFP-234	24*	14	0	15	93
MRFP-235	21.3	11	0	20	90
MRFP-236	23.3	28.6	12	15.6	100
MRFP-237	19.6	21.3	0	0	100
MRFP-238	24.6*	19.6	0	12.5	69.4
MRFP-239	25	29	14.6	0	61.5
MRFP-240	25.5	23.3	0	12.6	61.5
MRFP-241	30	20	0	15.5	70.9
MRFP-242	28	25	0	13.5	57
MRFP-243	NT	26.3	12.3	22.6	52.6

Table 2. (Cont'd.).

Pseudomonas isolates	*F. solani*	*F. oxysporum*	*R. solani*	*M. phaseolina*	Juvenile mortality
	Zone of inhibition (mm)			% After 48 hrs.	
MRFP-244	-	22.3	10.6	0	50
MRFP-245	-	30	0	18.3	43.7
MRFP-246	11.3	14	4.2	10.6	47.2
MRFP-247	12.6	14	13.3	16.6	85
MRFP-248	0	0	0	21.3	31.8
MRFP-249	15.3	13.3	NT	0	60
MRFP-250	NT	21.6*	6.6	NT	50
MRFP-251	-	24	20	-	43.7
MRFP-252	-	24.6	5	-	52
MRFP-253	-	21.6	0	-	76.6
MRFP-254	-	20	10	0	48
MRFP-255	-	29.3	0	NT	55.8
MRFP-256	-	20	0	-	57.1
MRFP-257	-	21.6	10	0	31.8
MRFP-258	-	20	7.5	-	60
MRFP-259	-	26.6	0	-	60
MRFP-260	25	0	0	-	50
MRFP-261	NT	0	0	-	55.8
MRFP-262	23.3	30.3	0	-	NT
MRFP-263	-	20	5	-	-
MRFP-264	-	23.3	6.6	-	-
MRFP-265	-	26.6	0	-	-
MRFP-266	-	18.3	0	-	-
MRFP-267	-	20	0	-	-
MRFP-268	-	20	5	-	-
MRFP-269	-	26.6	0	-	-
MRFP-270	-	17.6	0	-	-
MRFP-271	-	23.3	0	-	-
MRFP-295	0	30*	16	-	73.1
MRFP-304	16*	24	0	14*	79.7
MRFP-305	8*	28	18	9	87.1
MRFP-306	6	19*	21	9	70.6
MRFP-307	15*	13*	10	079.2	85.2
MRFP-308	10	16	0	6	74
MRFP-309	0	27*	8	0	
MRFP-310	9	24	0	3	74
MRFP-311	12	21	0	8	79.2
MRFP-312	8*	1	20	6	70
MRFP-313	18	18*	8	3	59.3
MRFP-314	3	0	11	7	67.6
MRFP-315	26	29*	7	16	76
MRFP-316	10	19	17	12	86
MRFP-317	12*	13	10	18*	82.4
MRFP-318	21	10	0	6	55

NT = Not tested, * Lysis of the fungal hyphae

Table 3. Effect of mycorrhizospheric fluorescent *Pseudomonas* (MRFP) on the infection of *Fusarium solani, F. oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina*on sunflower roots in screen house experiment.

Treatments	*F.solani*	*F. oxysporum*	*R. solani*	*M. phaseolina*
	Infection %			
Control	12.5	25	31.2	68.7
Control (*Glomus* sp.)	6.2	18.7	12.5	87.5
Carbendazim	12.5	6.2	6.2	75
MRFP-202	18.7	6.2	0	100
MRFP-203	12.5	6.2	12.5	81.2
MRFP-211	0	6.2	25	93.7
MRFP-212	18.7	18.7	0	87.5
MRFP-206	18.7	18.7	0	18.7
MRFP-202 + *Glomus* sp.	25	25	50	62.6
MRFP-203 + *Glomus* sp.	25	12.5	12.5	68.7
MRFP-206 + *Glomus* sp.	0	18.7	6.2	97.5
MRFP-211 + *Glomus* sp.	31.5	31.2	6.2	93.7
MRFP-212 + *Glomus* sp.	25	12.5	12.5	68.7
$LSD_{0.05}$	Treatment = 14.9[1], Pathogens = 6.4[2]			

[1]Mean values in column for treatments showing differences greater than LSD values are significantly different at $p<0.05$
[2]Mean values in rows for pathogens showing differences greater than LSD values are significantly different at $p<0.05$

Table 4. Effect of mycorrhizospheric fluorescent *Pseudomonas* (MRFP) on the growth of sunflower in a screen house experiment.

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. of VAM spore/g soil	Phosphorus (ppm)
Control	27.0	4.13	12.3	1.60	12.2	8.08
Control (carbendazim)	28.1	4.19	13.8	1.59	14.2	5.18
Glomus sp. (G)	25.5	4.0	12.8	1.35	28.1	13.3
MRFP-202	27.3	4.23	12.1	1.27	17.9	15.1
MRFP-203	28.4	4.15	4.5	1.35	21	13.8
MRFP-206	29.8	4.41	12.3	1.26	29	13.7
MRFP-211	30.5	4.6	13.8	2.76	24	10.1
MRFP-212	28.4	4.62	12.4	5.49	16	13.6
MRFP-202 + G	28.4	3.65	12.8	1.07	18.5	14.7
MRFP-203 + G	30.9	4.21	13.3	1.39	15.9	6.6
MRFP-206 + G	30.7	3.54	12.1	0.98	35.2	13.2
MRFP-211 + G	30.6	3.52	13.2	1.33	30.1	15.1
MRFP-212 + G	29.9	2.66	12.5	1.12	16.7	16.2
$LSD_{0.05}$	3.7[1]	ns	ns	3.35[1]	13.1[1]	1.76[1]

[1]Mean values in column for treatments showing differences greater than LSD values are significantly different at $p<0.05$

Table 5. Effects of mycorrhizospheric fluorescent *Pseudomonas* (MRFP) on infection of *Fusarium solani, F.oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* on sunflower roots in field experiment (2014).

Treatments	Infection %							
	F. oxysporum		*F. solani*		*R. solani*		*M. phaseolina*	
	30d	60d	30d	60d	30d	60d	30d	60d
Control	18.7	12.5	25	6.2	25	0	31.2	93.7
Control (carbendazim)	31.2	0	25	0	6.2	0	6.2	75
*P*seudomonas (MRFP-202)	18.7	6.2	18.7	0	31.2	0	18.7	81.2
Pseudomonas (MRFP-203)	25	0	43.7	0	6.2	0	18.7	93.7
Pseudomonas (MRFP-206)	25	6.2	31.2	0	50	6.2	43.7	56.2
Pseudomonas (MRFP-212)	6.2	0	50	0	18.7	0	25	75
$LSD_{0.05}$	Treatments= 9.6[1], Pathogens = 7.8[2], Days = 5.5[3]							

[1] Mean values in column for treatments showing differences greater than LSD values are significantly different at $p<0.05$
[2] Mean values in rows for pathogens showing differences greater than LSD values are significantly different at $p<0.05$
[3] Mean values in rows for days showing differences greater than LSD values are significantly different at $p<0.05$

Table 6. Effects of mycorrhizoshperic fluorescent *Pseudomonas* (MRFP) on the growth of sunflower in field experiment (2014).

Treatments	Shoot length (cm)		Shoot weight (g)		Root length (cm)		Root weight (g)		No. of VAM spores/g soil		Phosphorus (ppm)	
	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d
Control	17.9	106.4	5.7	41.38	7.1	9.7	1.09	5.17	18	10.8	-	3.75
Carbendazim	18.5	93.9	5.4	37.25	7.3	11.0	1.02	6.82	17.5	21.4	-	5.06
Pseudomonas (MRFP-202)	23.7	119.6	9.06	73.27	8.6	13.6	1.7	9.81	15.8	40.3	-	4.86
Pseudomonas (MRFP-203)	25.2	133.1	10.06	96.45	7.5	10.8	1.7	9.26	34.8	26	-	7.63
Pseudomonas (MRFP-206)	24.2	117.9	9.87	66.9	8.3	14.1	1.8	8.10	15.3	29.6	-	4.72
Pseudomonas (MRFP-212)	21.4	122.5	7.76	79.53	6.6	14.0	1.3	9.83	12.2	22.6	-	5.95
LSD (p<0.05)	5.52	0.3[1]	4.2[1]	34.8[1]	2.4[1]	3.27[1]	ns	ns	12.5[1]	16.9[1]	-	3.27[1]

[1] Mean values in column for treatments showing differences greater than LSD values are significantly different at p<0.05

Table 7. Effects of mycorrhizoshperic fluorescent *Pseudomonas* (MRFP) on infection of *Fusarium solani, F. oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* on sunflower roots in field experiment (2015)

Treatments	Infection %							
	F. oxysporum		*F. solani*		*R. solani*		*M. phaseolina*	
	30d	60d	30d	60d	30d	60d	30d	60d
Control	0	0	12.5	0	62.5	93.7	68.7	87.5
Control (carbendazim)	6.5	0	0	0	50	81.2	62.5	81.2
*P*seudomonas (MRFP-202)	0	0	18.7	0	31.2	31.2	50	56.2
Pseudomonas (MRFP-203)	0	0	0	0	31.2	62.5	56.2	62.5
Pseudomonas (MRFP-206)	6.5	0	12.5	0	50	62.5	43.7	75
Pseudomonas (MRFP-212)	0	0	6.2	0	75	68.7	25	50
$LSD_{0.05}$	Treatments = 27.1[1], Pathogens = 22.1[2], Days = 15.6[3]							

[1] Mean values in column for treatments showing differences greater than LSD values are significantly different at p<0.05
[2] Mean values in rows for pathogens showing differences greater than LSD values are significantly different at p<0.05
[3] Mean values in rows for days showing differences greater than LSD values are significantly different at p<0.05

Table 8. Effects of mycorrhizoshperic fluorescent *Pseudomonas* (MRFP) on the growth of sunflower in field experiment (2015).

Treatments	Shoot length (cm)		Shoot fresh weight (g)		Root length (cm)		Root weight (g)		No. of VAM spores/g soil		Phosphorus (ppm)	
	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d
Control	13.1	40.8	2.82	19.5	6.0	7.7	0.6	2.8	6.1	16.9	1.7	3.3
Carbendazim	13.3	57.4	2.84	46.3	5.6	10.5	0.58	2.5	7.4	15.7	2.8	2.3
Pseudomonas (MRFP-202)	15.9	62.1	3.95	30.5	5.8	10.3	078	5.3	24.9	26.9	2.4	4.4
Pseudomonas (MRFP-203)	15.9	60	3.34	42.3	7.0	11.4	0.7	5.7	17.9	21.7	3.7	5.3
Pseudomonas (MRFP-206)	13.3	42.4	2.47	26.4	5.4	9.4	0.6	2.3	27.5	27.7	5.12	5.4
Pseudomonas (MRFP-212)	13.7	53.5	3.23	34.2	5.5	11.0	07	4.9	12.2	27.6	8.7	5.7
LSD (p<0.05)	ns	2.9[1]	ns	4.3[1]	1.7[1]	ns	ns	ns	2.4[1]	2.6[1]	2.4[1]	0.6 1[1]

[1] Mean values in column for treatments showing differences greater than LSD values are significantly different at p<0.05 ns = nonsignificant

Discussion

As a result of raising awareness about the adverse effect of synthetic agrochemicals on the environment, biofertilizers are emerging as a suitable alternative of these chemicals, since they facilitate the growth and yield of crops in an eco-friendly manner (Basu *et al*., 2021). Plant growth promoting rhizobacteria (PGPR), particularly those belongs to fluorescent *Pseudomonas* are nonpathogenic, friendly bacteria, that stimulates plant growth by mitigating stress related damages and also by ameliorating the concentration of growth hormones and status of plant resistance markers (Moin *et al*., 2020; Turan *et al*., 2021; Urooj *et al*., 2021). In this study, fluorescent *Pseudomonas* (87 isolates) were isolated and identified from the mycorrhizosphere of healthy plants. Mycorrhizosphere is the region that occurs under the influence of both root exudates and mycorrhizal fungi (Johansson *et al.*, 2004). Most of these isolates caused growth inhibition of root rot fungi and killed root knot nematodes *In vitro*. Fluorescent *Pseudomonas* has been reported to produce antimicrobial compounds that suppress plant pathogens (Raaijmakers *et al*., 2002; Parveen *et al*., 2020a). Fluorescent *Pseudomonas* associated with rhizosphere, endophytic (Moin *et al.*, 2020; Korejo *et al.*, 2019), epiphytic (Habiba *et al*., 2016) and root nodules (Noreen *et al*., 2015; 2016) have been reported to suppress plant pathogenic fungi (Hol *et al*., 2013). In this study, better control of *F. oxysporum, F. solani,* and *R. solani* was found on sunflower when *Glomus* sp., was used with MRFP in screen house experiments. Fluorescent *Pseudomonas* are soil bacteria that colonize plant roots and reduce plant diseases via direct suppression of root rot pathogens (Rahman *et al.*, 2016).

The endo-mycorrhizal fungi are known to form association with plants and help their host in the uptake of nutrients, tolerance against stress, suppression of

soilborne plant pathogens, mobilization of minor elements and production of plant growth hormones (Basu & Santhaguru, 2009). However, mycorrhizospheric bacteria affect VAM fungi and their host plants (Johansson *et al*., 2004). The suppression of charcoal rot fungus (*M.phaseolina*) on sunflower is a very interesting outcome of this study. The charcoal rot fungus caused stem rot and root on plant species including sunflower (Kolte, 2018). Parveen *et al*., (2020b) reported charcoal rot disease of sunflower as a major problem in Sindh province of Pakistan. Suppression of charcoal rot of sunflower under field conditions is encouraging and advocating continuing the research on mycorrhizospheric fluorescent for the better management of soil-borne plant diseases.

In this study, application of mycorrhizospheric fluorescent *Pseudomonas* (MRFP) alone or mixed with VAM showed significant suppression of root rot pathogens of sunflower both in pots and field experiments and in some cases showed better plant growth. Most of the biofertilizers are known to perform well in the laboratory and greenhouse conditions but usually show inconsistent results or failed to deliver the expected effects on plant growth in field settings (Basu *et al.*, 2021)., which might be due to the different effect of MRFP on VAM. Meyer & Linderman, (1986) have reported that mycorrhizae associated bacteria have positive effect on mycorrhizal fungi, since they stimulate mycorrhizal activity. Majority of microorganisms associated with the mantle of ectomycorrhizae have positive effects on mycorrhizae were fluorescent *Pseudomonas* while few of them were neutral or inhibitory (Garbaye, 1994). Bokhari *et al*., (2013) reported stimulation of mycorrhizal activity by the mycorrhizospheric fluorescent *Pseudomonas* resulting in better uptake of phosphorus by the mungbean plant. The mycorrhizal association with plants is not bipartite, but it should include associated microorganisms (Tarkka & Frey-Klett, 2008), particularly fluorescent *Pseudomonas* (Scheublin *et al*., 2010, Lecomte *et al*., 2011). The role of mycorrhizospheric fluorescent *Pseudomonas* seems very important in plant-mycorrhizal symbiosis.

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