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# MANAGEMENT OF ROOT ROT AND ROOT KNOT DISEASE OF MUNGBEAN WITH THE APPLICATION OF MYCORRHIZOSPHERIC FLUORESCENT *PSEUDOMONAS* UNDER FIELD CONDITION

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**Abstract**

The mycorrhizosphere is the region around a mycorrhizal fungus in which nutrients released from the hyphae increases microbial population and its activities. In this study five mycorrhizospheric fluorescent *Pseudomonas* (MRFP) were evaluated for biocontrol potential under field condition using mungbean (*Vigna radiata*) as test plant. MRFP-249 significantly reduced *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina.* Whereas MRFP246 and MRFP-247 were also found effective against *M. phaseolina.* Mycorrhizospheric fluorescent *Pseudomonas* were also found effective against root knot nematode by reducing the galls on roots and nematode's penetration in roots. Highest fresh shoot weight and plant height was produced by MRFP-248. Plants grown in soil treated with *Pseudomonas* showed higher number of VAM spores around the mungbean roots than untreated control plants. The mycorrhizal symbiosis should not be considered merely as bipartite, plant-fungus interaction, but should instead include the associated microorganisms, particularly fluorescent *Pseudomonas.*

## Introduction

Soil microorganisms represent a spectrum from being harmful (pathogenic) to highly beneficial for plant growth and survival. A major component of these soil microorganisms is the complex of mycorrhizal fungi that colonize plant roots and adjacent soil (rhizosphere) at the same time, thus interact directly or indirectly with pathogenic microbes and plant growth promoting rhizobacteria (PGPR) (Sood, 2003). Mycorrhizal symbiosis generally increases root exudation and influences rhizosphere microbial communities. Mycorrhizal hyphae exude chemical compounds that have a selective effect on the microbial communities in the rhizosphere and in the soil. The zone under the influence of by both the root and the mycorrhizal fungus is termed as mycorrhizosphere (Johansson *et al*., 2004). There has been increasing evidence that the mycorrhizosphere communities have an important role in plant growth and soil fertility (Duponnois *et al.,* 2008). Vesicular arbuscular mycorrhizae (VAM) enhance plant growth through increased nutrient uptake, stress tolerance and disease resistance (Bouamri *et al.,* 2006; AlKaraki *et al.,* 2004; Pfleger & Linderman, 2000; Gamalero *et al.,* 2004; Siddiqui & Mahmood, 1995).

Several investigations have indicated that the rhizosphere bacteria have a strong impact on growth of VAM fungi (Deveau *et al.*,Linderman, 2000; Pivato *et al.,* 2008; Frey-Klett *et al.,* 2007). Positive interactions of plant growth promoting rhizobacteria and VAM fungi have been reported (Fitter & Garbaye, 1994; Sood, 2003). The PGPR have been reported to facilitate the colonization of plant by VAM fungi, improved the development of the mycosymbiont, and reduced the damage caused by soil-borne plant pathogens (Lumini *et al.,* 2007; Neeraj & Kanchan, 2010; 2011). *Paenibacillus* strain from the mycorrhizosphere of *Glomus mosseae* stimulated VAM colonization of plants by antagonizing soil-borne plant pathogenic fungi (Budi *et al.,* 1999). Root colonization of chickpea by *G. intraradices* was increased in the presence of *Pseudomonas putida* and *Paenibacillus polymyxa* (Akhtar & Siddiqui, 2007). Similarly mixed inoculation of *Glomus fasciculatum* and *Pseudomonas fluorescens* showed better control of *Bipolaris sorokiniana* on wheat and improved plant gowth than either component used singly (Hashemi *et al.,* 2013). The present report describes the role of mycorrhizospheric fluorescent *Pseudomonas* in suppressing the root rot and root knot diseases of mungbean and their effect on VAM population around the roots.

## Materials and Methods

The mycorrhizospheric fluorescent *Pseudomonas* (MRFP) used in this study were originally isolated from *Lycopersion esculentum* (MRFP-233) and *Triticum aestivum* (MRFP-246, MRFP-247, MRFP-248 and MRFP-249) and have shown significant antifungal and nematicidal activity (Bokhari *et al.,* 2013). The experiments were conducted at the Crop Diseases Research Institute, Pakistan Agricultural Research Council, Karachi University Campus, Karachi in 2X2 meter plots in complete randomized block design. The soil had a natural infestation of 3-14 sclerotia/g of soil of *Macrophomina phaseolina* (Sheikh & Ghaffar, 1975), 4-11% colonization of *Rhizoctonia solani* on sorghum

seeds used as baits (Wilhelm, 1955) and 2500cfu/gm of soil of mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution (Nash & Snyder, 1962). Seeds of mungbean (*Vigna radiata*) were sown at 50 seeds per two meter row and cell suspension of fluorescent *Pseudomonas* MRFP-233 (2.6 x $10^8$ cfu/ml), MRFP-246 (4.3 x $10^8$ cfu/ml), MRFP-247 (3.2 x $10^8$ cfu/ml), MRFP-248 (2.2 x $10^8$ cfu/ml) and MRFP-249 (4.2 x $10^8$ cfu/ml) were applied in each row at 200 ml. After germination each row was inoculated with *M. javanica* eggs/juveniles at 2000/ meter row. Carbendazim (200 ppm) 200 ml/ row served as positive control against root infecting fungi and carbofuran at 1.0g/ meter served as positive control against nematode. Each treatment was replicated four times and plants were watered 2-3 days intervals depending upon requirement of plants. Observations were recorded after 45 days of nematode inoculation. Incidence of root infecting fungi, root knot nematode, population of VAM spores around the roots and plant growth parameters were determined.

For the estimation of effect of fluorescent *Pseudomonas* on nematode infection, number of knots on each roots were recorded. To determine the nematode penetration, roots from each plant were cut into one cm long pieces and mixed thoroughly. One gram sub sample after washing in running tap water was wrapped in muslin cloth and dipped for 3-5 minutes in boiled 0.25% acid fuchsine stain. Roots were left in the stain to cool, and then washed under tap water to remove excess stain. Roots were transferred in vials containing 1:1 glycerol: water with few drops of lactic acid. Roots were macerated in an electric blender for 45 seconds and macerate suspended in 100 ml water. Number of $J_2$, $J_3$, $J_4$ and females in 5 samples of 5 ml each were counted with the aid of low power microscope and number of nematodes/g roots was calculated (Siddiqui *et al*., 2000). To determine the incidence of fungal infection, 1-cm long root pieces from tap roots (five pieces from each plant) were surface disinfested with 1% Ca $(OCl)_2$ solution and plated onto potato dextrose agar amended with penicillin (100,000 units/l) and streptomycin (0.2 g/l). After incubation for 5 days at 28°C, colonies of *Macrophomina phaseolina*, *Rhizoctonia solani* and species of *Fusarium* were recorded. Whereas population of VAM spores were determined using wet sieving and decanting technique (Gerdemann & Nicolson. 1963). The experiment was conducted twice. Data were subjected to analysis of variance (ANOVA) and means were separated using the least significant difference (LSD) according to Gomez & Gomez (1984).

**Results**

All the MRFP used caused a suppressive effect on *F.solani* infection. Whereas MRFP-249 caused a significant reduction in *F. solani*, *R. solani* and *M. phasolina* infection on mungbean roots. MRFP- 246 and MRFP-247 were also found effective *M. phaseolina* (Table 1). Plants grown in soil treated with MRFP-246 showed no infection of *F.solani*. MRFP also caused a suppressive effect on root knot nematode by reducing the galls on roots and nematode's penetration in roots (Table 2). MRFP-246, MRFP-247 and MRFP-249 besides, suppressing the *F. solani* and *M. phaseolina* also significantly ($p<0.05$) suppressed root knot infection. These *Pseudomonas* not only reduced the galls on roots but also greatly reduced nematode's penetration in roots as compared to control and carbofuran, a nematicide (Table 2). Plants grown in soil treated with *Pseudomonas* showed higher number of VAM spores around the roots than control plants (Table 2). Some isolates of fluorescent *Pseudomonas* also caused a positive effect on plant growth. Greater plant height was produced by MRFP-248 (Table 3).

**Table 1. Effects of mycorrhizoshperic fluorescent *Pseudomonas* (MRFP) on infection of *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* on mungbean in field experiment after 45 day of nematode inoculation.**

| Treatments | *F. solani* | *F. oxysporum* | *R. solani* | *M. phaseolina* |
|---|---|---|---|---|
| | | **Infection%** | | |
| Control | 50 | 18.7 | 25 | 37.5 |
| Carbendazim | 25 | 18.7 | 25 | 31.2 |
| Carbofuran | 12.5 | 0 | 12.5 | 6.2 |
| MRFP-233 | 25 | 6.2 | 25 | 31.2 |
| MRFP-246 | 0 | 18.7 | 18.7 | 6.2 |
| MRFP-247 | 6.25 | 25 | 31.2 | 12.5 |
| MRFP-248 | 31.2 | 31.2 | 25 | 25 |
| MRFP-249 | 12.5 | 12.5 | 6.2 | 6.2 |
| LSD0.05 | Treatments 13.09[1] | | Pathogens=9.2[2] | |

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

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

**Table 2. Effects of mycorrhizoshperic fluorescent *Pseudomonas* (MRFP) on the population of mycorrhizae in soil around the mungbean roots and infection of *Meloidogyne javanica,* the root knot nematode after 45 days of nematode inoculation in field experiment.**

| Treatments | No. of VAM spores/ gm soil | No. of Knots/ root system | Females/Juveniles per gram roots |
|---|---|---|---|
| Control | 17.7 | 24 | 119 |
| Carbendazim | 27.7 | 17.2 | 74.2 |
| Carbofuran | 27.5 | 15.5 | 62.7 |
| MRFP-233 | 39.7 | 10.2 | 33.7 |
| MRFP-246 | 40.7 | 12.5 | 28 |
| MRFP-247 | 42.2 | 11.5 | 23.7 |
| MRFP-248 | 36 | 11.5 | 23.2 |
| MRFP-249 | 39.7 | 15.7 | 31.7 |
| LSD0.05 | 3.6[1] | 3.0[1] | 5.5[1] |

[1]Mean values in the column showing difference greater than LSD value are significantly different at $p<0.05$

**Table 3.Effects of mycorrhizoshperic fluorescent *Pseudomonas* (MRFP) on the growth of mungbean plants in field experiment after 45 days of nematode inoculation.**

| Treatments | Shoot length (cm) | Shoot weight (g) | No. of fruit/plant | Fruit wt. (g) |
|---|---|---|---|---|
| Control | 52.0 | 16.9 | 0 | 0 |
| Carbendazim | 63.6 | 15.6 | 3.5 | 3.02 |
| Carbofuran | 77.7 | 19.1 | 5 | 4.1 |
| MRFP-233 | 58.7 | 17.2 | 3 | 3 |
| MRFP-246 | 64.9 | 21.7 | 0 | 0 |
| MRFP-247 | 53.7 | 16.1 | 2 | 1.13 |
| MRFP-248 | 87.4 | 24.9 | 2.5 | 1.19 |
| MRFP-249 | 60.1 | 21.7 | 1 | 1.02 |
| LSD0.05 | 26.0[1] | ns | ns | ns |

[1]Mean values in column showing differences greater than LSD values a re significantly different at $p<0.05$
ns = non-significant

**Discussion**

The mycorrhizosphere is the region around a mycorrhizal fungus in which nutrients released from the fungal hyphae increased the microbial population and its activities (Timonen & Marschner, 2006). In this study application of fluorescent *Pseudomonas* isolated from mycorrhizosphere caused suppression of *Macrophomina phaseolina* and *Fusarium solani* infection on mungbean. The root colonizing bacteria that have a beneficial effect on plants are termed as plant growth promoting rhizobacteria (Kloepper *et al*., 1980) have been reported to improve plant growth either through direct stimulation of the plant by producing growth regulators or by suppression of pathogens (Brown, 1972; Weller *et al*., 2002; Raaijmakers *et al*., 2002; Inam-ul-Haq *et al.,* 2012). Of the various rhizospheric bacteria, the bacteria belonging to fluorescent *Pseudomonas*, which colonize roots of a wide range of crop plants, are also reported to be antagonistic to soilborne plant pathogens (Izhar *et al*., 1995; Ehteshamul-Haque *et al.,* , 1997ab; Siddiqui *et al*., 2000; Siddiqui & Ehteshamul-Haque, 2001). Similarly protection of plant roots from the attack of soilborne plant pathogens by the mycorrhizal fungi is well documented (Johansson *et al.,* 2004; Habte *et al.,* 1999; Linderman, 2000; Akhtar & Siddiqui, 2007; 2008; Neeraj & Kanchan, 2010). In the present study mycorrhizospheric fluorescent *Pseudomonas* also caused significant nematicidal effect on *Meloidogyne javanica* by reducing the nematodes gall on roots and nematodes penetration in roots. The colonization of roots by rhizosphere bacteria has also been reported to reduce nematode infection (Ehteshamul-Haque *et al.,* 2007a). *Meloidogyne incognita* gall on tomato, cucumber and clover was suppressed following application of bacterial soil drenches or root treatment (Zavaleta-Mejia & Van Gundy, 1982). Similarly, *Pseudomonas aeruginosa* has been reported to suppress root knot infection on chili, watermelon, guar and pumpkin (Parveen *et al*., 1998) and on tomato (Siddiqui & Ehteshamul-Haque, 2001).

In this study, application of mycorrhizosphereic fluorescent *Pseudomonas* significantly increased VAM population around the roots and improved plant growth.

An increased VAM population around the roots and better uptake of phosphorus by mungbean plants by the mycorrhizospheric fluorescent *Pseudomonas* has been reported (Bokhari *et al*., 2013). The interactions between soil bacteria and VAM fungi has been reported both negative and positive (Meyer & Linderman, 1986; Secilia & Bagyaraj, 1987), however, generally these interactions are synergistic, which includes stimulation of root colonization by mycorrhizal fungi (Bagyaraj & Menge, 1978; Chanway & Holl, 1991). Neeraj & Kanchan (2011) reported the reduction of root rot disease and increased in yield of *Phaseolus vulgaris* by the mixed inoculation of mycorrhizae and *P.fluorescens.* Our study suggests that mycorrhizospheric fluorescent *Pseudomonas* not only suppressed the root rotting fungi and root knot nematodes, but also stimulates the growth and proliferation of VAM fungi. The VAM fungi are well known for their role in uptake of nutrients by plants specially phosphorus and biological control of soilborne plant pathogens.

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