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ROLE OF RHIZOBIA IN SUPPRESSING THE ROOT ROT AND ROOT KNOT DISEASE OF CHILI USED ALONE OR WITH *PSEUDOMONAS AERUGINOSA*

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Abstract

In this study biocontrol potential of rhizobia individually and in combination with *Pseudomonas aeruginosa* were evaluated against root rot and root-knot diseases of chilies. *In vitro* seven rhizobial isolates were tested, NFB-30 (*Sinorhizobium sahelens*) inhibited growth of all four test fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium oxysporum* and *F. solani* and produced zone of inhibition of 10, 2, 2, and 11 mm respectively. Similarly seven isolates of *Pseudomonas aeruginosa* tested, PGPR-4, PGPR-6 and PGPR-11 suppressed the radial growth of all four fungi tested. In screen house experiments PGPR-6, PGPR-8, PGPR-11 and PGPR-37 showed significant decrease in infection of root rot fungi in chili. While NFB-28, NFB-30, NFB-31 & NFB-32 rhizobial isolates were found effective. In field experiments co-inoculation of different strains of PGPR with rhizobia showed better control of root rotting fungi and root knot nematode on chili than either component alone.

Key words: Rhizobia; Root rot; Root knot; Chili; *Pseudomonas aeruginosa*.

Introduction

Chili (*Capsicum annuum* L.) is an annual herbaceous vegetable and spice grown in both tropical and subtropical areas and consumed as fresh or processed. It is a cash crop, making Pakistan among the important producer of chili in the world (Iqbal *et al.,* 2010ab). In 2014-15, Pakistan exported US$ 4.7 million of chilies (Anon., 2016). However, many factors endangered agricultural sustainability of chilli, besides aflatoxin contamination (Sahar *et al.,* 2017).

Mostly diseases are resulted in lower quality and marketability of fruit which causes economic losses (Paterson, 2007, Iqbal *et al.,* 2010ab). The chili wilt, caused by root rotting fungi, *Fusarium* spp., *Rhizoctonia solani* and *Phytophthora capsici* has been reported as the most commonly known disease problem (Skaggs *et al.,* 2000; Sanogo, 2003). In Pakistan species of *Fusarium* like *F. oxysporum* and *F. solani* are most common species linked with diseased chili crop (Ehteshamul-Haque & Ghaffar, 1994; Qureshi *et al*., 2004). The relation of *Fusarium* species with *R. solani* or root knot nematodes *(Meloidogyne* spp.) is causing vast losses in Sindh, Pakistan (Parveen, 2020; Tariq *et al*., 2009).

Use of beneficial organisms such as bio-control agents (Urooj *et al.,* 2018) are considered for eco-friendly approaches and had been practiced since long time to improve soil quality, plant health and ultimately crop yield (Anon., 2011; Noreen *et al.,* 2018a, 2019). These beneficial microorganisms live in the rhizosphere, a narrow zone of soil under the influenced of root system and utilized the plant root exudates and increased plant growth by nitrogen fixation, siderophore, phytohormones production and phosphate solubilization (Rahman *et al*., 2017; Sridevi & Mallaiah, 2009; Noreen *et al*., 2018b). They also protected the plants by activating induced systemic resistance against pathogens and pests (Rahman *et al*., 2016; Korejo *et al.,* 2019).

Rhizobium spp., are soil inhabiting bacteria that established a symbiotic association with leguminous plants are also known to inhibit the soilborne phytopathogens (Deshwal *et al.,* 2003) such as *M. phaseolina*, *R. solani* (Omar & Abd-Alla, 1998), *Fusarium* spp., (Omar & Abd-Alla, 1998; Noreen *et al.,* 2015) and *Pythium ultimum* (Ozkoc & Deliveli, 2001). In our previous studies, rhizobia significantly suppressed root rot disease caused by *M. phaseolina*, *R. solani* and *Fusarium* spp., naturally infested with these pathogens on both leguminous and non-leguminous plants in screen house and under field conditions (Ehteshamul-Haque & Ghaffar, 1993, Noreen *et al.,* 2015; 2016).

Another bacteria belongs to fluorescent *Pseudomonas* spp., are known as antagonistic rhizospheric colonizers of different crop plants and have shown significant action against soilborne plant pathogens (Noreen *et al.,* 2015; Korejo *et al.,* 2017; Siddiqui & Ehteshamul-Haque, 2001; Siddiqui *et al.,* 2000; Weller *et al.,* 2002). Combined application of Rhizobia and PGPR has a synergistic outcome on bean growth and use of PGPR may increase efficiency of *Rhizobium* for common bean production (Korir *et al*., 2017). The current study defines the biocontrol potential of rhizobia and PGPR against root rotting fungi and root knot nematode infecting chili roots in screen house and under field conditions.

Materials and Methods

Bacterial cultures: Bacterial cultures (rhizobia and *Pseudomonas aeruginosa*) used in this study were obtained from culture collection of Department of Botany, University of Karachi that have shown significant nematicidal and fungicidal activity in our previous study (Ehteshamul-Haque *et al.,* 2007; Parveen *et al*., 2008).

***In vitro* nematicidal and fungicidal effects of cell free culture filtrates of *Pseudomonas aeruginosa* and rhizobia:** Bacterial isolates were grown in King's B (for

PGPR) or Yeast extract mannitol (for rhizobia) broths at 30ºC for 48 hours in dark and centrifuged twice at 3000 rpm for 20 minutes. The pellets were discarded and supernatants were collected in a beaker. The filtrates were then exposed to chloroform vapours for 1 minute and then allow to evaporate under laminar flow hood. Control broths were also exposed to chloroform vapors in the similar way.

Nematicidal activity: One ml cell free culture filtrate of bacterial strains and 1 ml of second stage juvenile suspension (25-40 juveniles) were transferred in glass cavity blocks and kept at 26 ±5ºC. Sterile respective broth were served as control There were three replicates of each treatment and juvenile mortality was recorded after 24 and 48 hours. The experiment was repeated twice.

Fungicidal activity: Fungicidal activity of cell free culture filtrates of bacterial strains were examined by agar disc diffusion method. Filter paper discs were loaded with filtrates at 20 µl/disc and dried. The loaded discs were placed at one side of the petri dishes containing Czapek'sDox agar (pH 7.2). A 5mm disc of actively growing culture of plant pathogens like *F. solani, F. oxysporum, R. solani* and *M. phaseolina* were inoculated at another side of the dish. Petri dishes were incubated at 28ºC for 7 days. Distance from fungal colony and disc was considered as zone of inhibition and measured in mm and averaged. The experiment was repeated twice with 3 replicates.

Screen house experiment

Effect of *P. aeruginosa* on root diseases and growth of chili plants: Three weeks old, equal sized chili seedlings were used in this experiment, raised in steam autoclaved sandy loam soil in 35-cm diam. earthen pots with surface sterilized seeds with 1% $Ca(OCl)_2$. *P. aeruginosa* strains grown on King's B medium at room temperature (25-30ºC) for 3 days, were scrapped with sterilized spatula after adding 10 ml sterilized 0.5% gum arabic in each plate and collected in a beaker. Roots of seedlings were dipped in each *P. aeruginosa* strain suspension viz., PGPR-4 (5.4 x 10^8cfu/ml), PGPR-5 (5.8 x 10^8cfu/ml), PGPR-6 (6.0 x 10^8cfu/ml), PGPR-8 (8.6 x 10^8cfu/ml), PGPR-11 (5.2 x 10^8cfu/ml), PGPR-27 (8.0 x 10^8cfu/ml), PGPR-37 (3.8 x 10^8cfu/ml), and PGPR-43 (5.0 x 10^8cfu/ml) for 1 hour, then transplanted in 10 cm diam. plastic pots, each containing 350 g soil. The soil was naturally infested with *M. phaseolina* (2-8 sclerotia g^{-1} of soil) as determined by wet sieving and dilution plating (Sheikh & Ghaffar, 1975), 3-10% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu g^{-1} of soil of a mixed population of *F. oxysporum* and *F. solani* as determined by soil dilution technique (Nash & Snyder, 1962). Seedlings treated with 0.5% gum arabic served as control. The experiment was conducted with 4 replicates and pots were randomized on a screen house bench. Observations were record after 30 days of growth. Infection by root infecting fungi was determined in the laboratory by isolating on PDA plates. Data on plant growth (like fresh weight of shoot and roots, plant height and root length) and nematode infection were also recorded. Nematode's penetration in roots was determined by placing I g infected roots in boil acid fushion stain and then grinded 50 ml of water in an electric grinder. Nematode's juveniles/females were counted under steromicroscope (Siddiqui *et al.,* 2000).

Effect of rhizobial isolates on root diseases and growth of chili plants:. In this experiment 3 weeks old chili seedlings of equal size were used, Rhizobial strains grown on YEMA medium at room temperature (25-30ºC) for 3 days were scraped with the help of a sterilized bent spatula after adding 10ml sterilized 0.5% gum arabic in each plate and pooled in beakers. Roots of seedling were dipped in each rhizobial suspension viz., NFB-1 (*Bradyrhizobium* spp.) (6.2 x 10^8cfu/ml), NFB-2 (*Rhizobium* spp.) (6.0 x 10^8cfu/ml), NFB-28 (*S. meliloti*) (7.5 x 10^8cfu/ml), NFB-29 (*S. meliloti*)(9.0 x 10^8cfu/ml), NFB-30(*S. sahelens*) (7.0 x 10^8cfu/ml), NFB-31 (*S. sahelens*) (5.2 x 10^8cfu/ml) and NFB-32.(*S. sahelens*) (5.0 x 10^8cfu/ml) for 1 hour, then transplanted in 8cm diam plastic pots, each containing 300g soil. Seedlings treated with 0.5% gum aerobic served as control. There were 4 replicates of each treatment and pots were randomized on a screen house bench. Observations were recorded after 30 days growth.

Field experiment: *Pseudomonas aeruginosa* strains PGPR-37, PGPR-11 and PGPR-6 multiplied on King's B medium and rhizobial strains NFB-28 and NFB-30 on YEMA at 28ºC were used in this experiment. Other details are same as described in pots experiments. Chili roots were dipped in aqueous-gum-arabic suspension of PGPR-37 (2.5 x 10^8cfu/ml), PGPR-11 (1.2 x 10^8cfu/ml) PGPR-6 (1.0 x 10^8cfu/ml) NFB-28 (*S. meliloti*) (3.5 x 10^8cfu/ml), NFB-30 (*S. sahelens*) (2.7 x 10^8cfu/ml) for 1 hour and transplanted in 2 x 2 meters field plots at the experimental field of Crop Diseases Research Institute, Pakistan Agricultural Research Council, Karachi, University Campus at 12 seedlings per row. The soil had a natural infestation of 6-18 sclerotia /g of soil of *M. phaseolina*, 5-12% colonization of *R. solani* on sorghum seeds used as baits and 2800cfu/g of soil of mixed population of *F. oxysporum* and *F. solani*. Each treatment was replicated four times. Plants were watered according to the requirement of plants, 5 days after the establishment of seedlings, 10 handpicked or selected egg masses of *M. javanica* were inoculated around each seedling. Plants were uprooted after 45 and 75 days (4 plants per replicate; total 16 per treatment) of nematode inoculation.

Results

Nematicidal activity of PGPR and rhizobia: Cell free culture filtrates of PGPR and rhizobia caused significant nematicidal activity by killing nematode larvae at varying degrees. PGPR-4, PGPR-5, PGPR-11, PGPR-27, PGPR-37 and PGPR-43 killed more than 50% nematode larvae within 24 hours. The nematicidal activity increased with an increase in exposure time. PGPR-4, PGPR-11 and PGPR-37 caused more than 80% nematode mortality after 48 hrs (Table 1).

Cell free culture filtrates of rhizobial strains also showed significant nematicidal activity. NFB-1 (*Bradyrhizobium* spp.), NFB-2 (*Rhizobium* sp.), NFB-28 (*S. meliloti*), NFB-30 (*S. sahelens*), NFB-31 (*S. sahelens*), and NFB-32 (*S. sahelens*), caused more than 50% larval mortality within 48 hours (Table 1).

Table 1. Nematicidal activity or cell free culture filtrates of *Pseudomonas aeruginosa* and rbizobial isolates against *Meloidogyne javanica*.

Treatments	Juveniles mortality %	
	24 h	48 h
P. aeruginosa		
Control	0	10
PGPR-4	60	80
PGPR-5	55	75
PGPR-8	40	65
PGPR-11	70	90
PGPR-27	65	75
PGPR-37	65	80
PGPR-43	60	75
***Rhizobial* isolates**		
Control	00	10
Bradyrhizobium sp. (NFB-1)	30	64
Rhizobium sp. (NFB-2)	25	63
S. meliloti (NFB-28)	15	50
S. meliloti (NFB-29)	15	45
S. sahelens (NFB-30)	45	70
S. sahelens (NFB-31)	25	60
S. sahelens (NFB-32)	20	62

Fungicidal activity of PGPR and rhizobia: Cell free culture filtrates of PGPR-4, PGPR-5 and PGPR-11 caused growth suppression of all the four test fungi by producing zone of inhibition of 6.5, 2 and 6 mm against *M. phaseolina*, 5.5, 2 and 2.3 mm against *R. solani*, 2.5, 2.3 and 3.2 mm against *F. oxysporum* and 14.5, 10 and 10 mm against *F. solani* respectively (Table 2). Whereas, out of seven rhizobial isolates tested, NFB-30 (*S. sahelens*) inhibited all the four test fungi and produced a zone of inhibition of 10, 2, 2 and 11 mm against *M. phaseolina, R. solani, F. oxysporum* and *F. solani* respectively (Table 2). While, other strains inhibited at least one pathogen.

Screen house experiment (PGPR): Observations were recorded after 30 days showed that *P. aeruginosa* PGPR-37 and PGPR-43 significantly ($p<0.05$) reduced *M. phaseolina* infection on chili roots (Table 3). Less infection of *F. solani* was observed in most of the treatments except PGPR-8, whereas *P. aeruginosa* strains PGPR-4, PGPR-5, PGPR-11, PGPR-27, PGPR-37 and PGPR-43 were used. *P. aeruginosa* strains PGPR-4, PGPR-5, PGPR-11, PGPR-37 and PGPR-43 were found highly effective against *R. solani* by completely suppressing its infection. Complete suppression of *F. oxysporum* infection was produced by PGPR-37and PGPR-43 (Table 3). Greater plant height was produced by PGPR-43 followed by PGPR-37. Whereas, maximum fresh weight of shoot was produced by PGPR-37(Table 3).

Screen house experiment (Rhizobia): Observations were recorded after 45 days, showed that rhizobial strains NFB-30 (*S. sahelens*), NFB-31 (*S. sahelens*) and NFB-32 (*S. sahelens*) completely prevented the *F. solani* infection, whereas rhizobial strain NFB-28 (*S. meliloti*) also significantly ($p<0.05$) reduced *F. solani* infection on chili roots. Infection% of *M. phaseolina* and *F. oxysporum* were significantly ($p<0.05$) controlled by NFB-1 (*Bradyrhizobium* sp.), NFB-2 (*Rhizobium* sp.), NFB-28 (*S. meliloti*), NFB-29 (*S. meliloti*), NFB-30 (*S. sahelens*), NFB-31 (*S. sahelens*) and NFB-32 (*S. sahelens*) (Table 4).

Rhizobial strains caused inhibitory effect on root knot nematode by reducing the number of galls and nematode penetration in roots (Table 5). Greater plant height was produced by NFB-29 (*S. meliloti*), and NFB-32 (*S. meliloti*), whereas NFB-1 (*Bradyrhizobium* spp.), NFB-2 (*Rhizobium* sp.), NFB-28 (*S. meliloti*), NFB-29 (*S. meliloti*), NFB-30 (*S. sahelens*), NFB-31 (*S. sahelens*) and NFB-32 (*S. sahelens*), also significantly ($p<0.05$) increased fresh shoot weight (Table 5).

Table 2. *In vitro* growth inhibition of root infecting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani, F.oxysporum* and nematicidal activity by cell free culture filtrates of *Pseudomonas aeruginosa* and rhizobial isolates.

Treatments	Zone of inhibition (mm)				Juveniles mortality %	
	M. phaseolina	*R. solani*	*F. oxysporum*	*F. solani*	24 h	48 h
Control	0	0	0	0	0	10
P. aeruginosa						
PGPR-4	6.5	5.5	2.5	14.5	60	80
PGPR-6	2	2.0	2.3	10	55	75
PGPR-8	0	0	1.5	10	40	65
PGPR-11	6	2.3	3.2	10	70	90
PGPR27	5	1.5	**	12.3	65	75
PGPR-37	0	*	**	6.5	65	80
PGPR-43	5	*	1.5	11.5	60	75
Rhizobial isolates						
Bradyrhizobium sp.(NFB-1)	5	*	**	5	30	64
Rhizobium sp. (NFB-2)	2.0	*	0	6.0	25	63
S. meliloti (NFB-28)	0	0	0	8.3	15	50
S. meliloti (NFB-29)	0	*	0	3	15	45
S. sahelens (NFB-30)	10	1.5	1.5	11.5	45	70
S. sahelens (NFB-31)	*	*	1.5	10.5	25	60
S. sahelens (NFB-32)	*	*	*	10.5	20	62

*No inhibition

**Growth inhibited but no zone was formed

Table 3. Effect of *Pseudomonas aeruginosa* on the infection of *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani* and growth of chili in screen house experiment.

Treatments	Infection %				Plant height (cm)	Fresh shoot weight (g)
	M. phaseolina	*R. solani*	*F. oxysporum*	*F. solani*		
Control	37.5	81.0	62.5	12.5	6.7	0.50
PGPR-4	43.7	25.0	00.0	06.2	6.7	0.57
PGPR-5	56.2	56.2	00.0	00.0	6.1	0.5
PGPR-6	12.5	43.7	00.0	00.0	7.2	0.55
PGPR-8	25.0	50.0	00.0	25.0	6.6	0.56
PGPR-11	31.2	18.7	00.0	06.2	6.8	0.54
PGPR-27	31.2	18.7	43.7	00.0	5.7	0.67
PGPR-37	18.7	06.2	00.0	00.0	7.5	2.20
PGPR-43	06.2	56.2	00.0	00.0	7.9	0.70
$LSD_{0.05}$	Treatments = 17.8[1]; Pathogens = 8.0[2]				0.9[1]	0.38[1]

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

Table 4. Effect of rhizobial isolates on the infection of *Macrophomina phaseolina*, *Fusarium oxysporum* and *F. solani* on chili roots.

Treatments	Infection %		
	M. phaseolina	*F. solani*	*F. oxysporum*
Control	50	87.5	50
NFB-1 (*Bradyrhizobium* spp.)	25	62.5	25
NFB-2 (*Rhizobium* sp.)	25	62.5	25
NFB-28 (*S. meliloti*)	12.5	31.2	12.5
NFB-29 (*S. meliloti*)	25	81.2	25
NFB-30 (*S. sahelens*)	25	0	25
NFB-31 (*S. sahelens*)	6.2	0	6.2
NFB-32 (*S. sahelens*)	6.2	0	6.2
$LSD_{0.05}$	Treatments = 16.4[1], Pathogens= 10.0[2]		

[1]Mean values for treatments in columns showing difference of LSD values are significantly different at $p<0.05$
[2]Mean values for pathogens in rows showing difference of LSD values are significantly different at $p<0.05$

Table 5. Effect of rhizobial isolates used as seedling treatment on the suppression of *Meloidogyne javanica* and growth of chili plants.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Galls/ root system	Juveniles & female/ g root
Control	7.99	0.24	3.37	17.49
NFB-1 (*Bradyrhizobium* sp.)	8.15	0.50	1.98	4.41
NFB-2 (*Rhizobium* sp.)	8.66	0.52	3.0	10.16
NFB-28 (*S. meliloti*)	8.52	0.55	1.93	2.24
NFB-29 (*S. meliloti*)	9.75	0.62	1.51	3.24
NFB-30 (*S. sahelens*)	8.80	0.43	1.80	2.15
NFB-31 (*S. sahelens*)	8.29	0.63	2.48	5.64
NFB-32 (*S. sahelens*)	9.35	0.63	3.25	7.41
$LSD_{0.05}$	1.4[1]	0.19[1]	1.27[1]	5.2[1]

[1]Mean values for treatments in columns showing difference of LSD values are significantly different at $p<0.05$

Table 6. Combined effect of *Pseudomonas aeruginosa* and rhizobial isolates used as seedling treatment on the infection of *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium oxysporum* and *F. solani* on chili roots under field condition.

Treatments	Infection %							
	F. solani		*F. oxysporum*		*M. phaseolina*		*R. solani*	
	45days	75days	45days	75days	45days	75days	45days	75days
Control	87.5	31.2	25	25	56.2	68.7	12.	0
PGPR-6	12.5	0	18.7	6.2	56.2	50	0	0
PGPR-37	31.2	6.2	18.7	12.5	62.5	50	0	0
PGPR-11	25	18.7	0	18.7	37.5	31.2	0	0
NFB-28 (*S. meliloti*)	18.7	0	18.7	18.7	50	25	0	6.2
NFB-30 (*S. sahelens*)	31.2	0	12.5	12.5	37.5	50	0	0
PGPR-37+ PGPR11	12.5	0	12.5	6.2	18.7	100	0	0
PGPR-37+ NFB-28	0	0	6.2	18.7	12.5	81.2	0	0
PGPR-37+ NFB-30	6.2	6.2	12.5	0	18.7	81.2	0	0
PGPR-37+ PGPR-6	0	6.2	0	6.2	12.5	81.2	0	0
PGPR-11 + NFB-28	31.2	0	0	0	12.5	75	6.2	0
PGPR-11 + NFB-30	31.2	6.2	12.5	6.2	43.7	56.2	12.5	0
PGPR-11 + PGPR-6	18.7	12.5	6.2	6.2	43.7	0	0	12.5
NFB-28 + NFB-30	18.7	12.5	0	0	12.5	0	0	31.2
NFB-28 + PGPR-6	43.7	25	18.7	0	12.5	0	0	6.2
NFB-30 + PGPR-6	31.2	25	0	0	18.7	0	0	0
$LSD_{0.05}$	Treatment = 11.13[1]; Pathogens = 5.56[2]; Time = 3.93[3]							

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

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

[3]Mean values for time in rows showing differences greater than the LSD value are significantly different at $p<0.05$

Table 7. Combined effect of *Pseudomonas aeruginosa* and rhizobial isolates used as seedling treatment on the infection of *Meloidogyne javanica* and growth of chili plants under field condition.

Treatments	Plant height (cm)		Fresh shoot weight (g)		Galls/root system		Juvenile & female/g root	
	45 days	75 days	45 days	75 days	45 days	75 days	45 days	75 days
Control	21.5	32.23	12.68	45.0	7.37	65.5	37.5	141.25
PGPR-6	30.0	64.8	31.8	131	2.75	11.5	7.0	6.75
PGPR-37	23.66	30.48	25.49	63.3	10.0	38.65	19.5	66.25
PGPR-11	23.0	17.93	23.5	10.0	7.3	2.56	19.0	10.75
NFB-28 (*S. meliloti*)	22.68	36.63	18.01	59.46	2.81	6.87	9.0	12.25
NFB-30 (*S. sahelens*)	25.5	47.0	27.59	60.0	3.0	9.18	9.0	12.0
PGPR-37 + PGPR11	18.8	52.2	7.09	58.0	4.9	23.68	37.5	25.0
PGPR-37 + NFB-28	30.12	55.8	22.0	132.4	4.75	31.75	18.0	65.0
PGPR-37 + NFB-30	26.75	48.17	22.56	92.3	4.4	3.56	16.5	11.0
PGPR-37 + PGPR-6	28.43	53.4	34.0	63.22	8.6	23.23	31.25	38.0
PGPR-11 + NFB-28	26.0	38.18	15.18	37.0	4.5	21.12	12.5	15.0
PGPR-11 + NFB-30	36.0	68.43	22.7	96.2	3.5	3.80	12.5	15.25
PGPR-11 + PGPR-6	21.69	40.6	15.5	58.4	2.8	23.12	10.25	50.25
NFB-28 + NFB-30	22.68	61.0	21.7	111.75	4.6	39.0	15.5	121.75
NFB-28 + PGPR-6	22.18	49.34	15.13	56.0	6.37	55.68	21.75	128.7
NFB-30 + PGPR-6	23.12	45.0	16.29	79.0	7.57	60.0	26.25	121.25
$LSD_{0.05}$	13.17[1]	18.46[1]	19.51[1]	70.9[1]	6.81[1]	28.7[1]	4.54[1]	25.79[1]

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

Field experiment: *Fusarium solani* infection was completely controlled by PGPR-37+ NFB-28 (*S. meliloti*), PGPR-37 + PGPR-6 after 45 days, but after 75 days it was completely controlled by NFB-28 (*S. meliloti*), NFB-30 (*S. sahelens*), PGPR-6, PGPR-37 + PGPR-11, PGPR-37 + NFB-28 (*S. meliloti*) and PGPR-11 + NFB-28 (*S. meliloti*) while *F. solani* infection was reduced after 45 days significantly ($p<0.05$) by PGPR-37, PGPR-11, PGPR-37 + NFB-30 (*S. sahelens*), PGPR-37 + PGPR-6 and PGPR-11 + NFB-30 (*S. sahelens*). *Fusarium oxysporum* infection was significantly ($p<0.05$) reduced by PGPR-6, PGPR-37 + PGPR-11, PGPR-37 + PGPR-6, PGPR-11 + NFB-30 (*S. sahelens*), PGPR-11 + PGPR-6 and was completely controlled by PGPR-37 + NFB-30 (*S. sahelens*), PGPR-11 + NFB-28 (*S. meliloti*) NFB-28 (*S. meliloti*) + NFB-30 (*S. sahelens*), NFB-28 (*S. meliloti*) + PGPR-6, NFB-30 (*S. sahelens*) + PGPR-6. Infection of *M. phaseolina* was completely suppressed by PGPR-11 + PGPR-6, NFB-28 (*S. meliloti*) + NFB-30 (*S. sahelens*), NFB-28 (*S. meliloti*) + PGPR-6, NFB-30 (*S. sahelens*) + PGPR-6. It was significantly ($p<0.05$) controlled by PGPR-11, and NFB-28 (*S. meliloti*) after 75 days. *Rhizoctonia solani* infection was completely controlled by PGPR-37, PGPR-11, NFB-28 (*S. meliloti*), NFB-30 (*S. sahelens*), PGPR-6, PGPR-37 + PGPR-11, PGPR-37 + NFB-30 (*S. sahelens*), PGPR-37 + PGPR-6, PGPR-11 + PGPR-6, NFB-28 (*S. meliloti*) + NFB-30 (*S. sahelens*), NFB-28 (*S. meliloti*) + PGPR-6 and NFB-30 (*S. sahelens*) + PGPR-6 (Table 6).

Plant height was significantly ($p<0.05$) increased by PGPR-11 + NFB-30 (*S. sahelens*) while the fresh weight of plant significantly ($p<0.05$) increased by PGPR-6, PGPR-37 + NFB-28 (*S. meliloti*), PGPR-37 + NFB-30 (*S. sahelens*), PGPR-11 + NFB-30 (*S. sahelens*) and NFB-28 (*S. meliloti*) + NFB-30 (*S. sahelens*) (Table 7).

Discussion

In current research, cell free culture filtrates of *P. aeruginosa* and rhizobia caused growth suppression of root rotting fungi and root knot nematode. El-Hadad *et al.,* (2011) reported that bacterial culture inhibited the infection of *Meloidogyne* spp., and also increased the growth of tomato There are several studies regarding the secretion of metabolites by rhizosphere bacteria that caused lysis of nematode eggs (Westcott & Kluepfel, 1993), decreases egg hatching (Oostendorp & Sikora, 1990), affects the survival of second stage juveniles (Noreen *et al.,* 2015) and damages root exudates, attractive to nematodes, causing the reduction of nematode attraction to roots and penetration (Oostendorp & Sikora, 1990). Similarly rhizobia that are known to produce biocide were also found to inhibit root knot nematodes infection on mungbean (Noreen *et al.,* 2015), okra and tomato (Parveen *et al*., 1993) and chickpea (Noreen *et al.,* 2016).

In this study, out of 7 cell free culture filtrates of *P. aeruginosa* strains used, 3 showed more than 80% mortality of *M. javanica* juveniles after 48 hours exposure. Whereas, out of 7 cell free culture filtrates of rhizobial isolates tested, 6 caused more than 50% larval mortality of *M. javanica* within 48 hours .Hashem & Abo-Elyousr (2011) found 45% mortality of *M. incognita In vitro*. Parveen *et al*., (2019) reported that rhizobial isolates not only increase the growth of soybean but also inhibited the *Meloidogyne* spp.

In the present study, *P. aeruginosa* and rhizobial strains used as seedling treatment significantly prevented the infection of *M. phaseolina*, *R. solani*, *F. oxysporum* and *F. solani* on chili plants. Plant growth promoting rhizobacteria colonized plant roots and have a positive effect on plants (Kloepper *et al*., 1980). They improved growth of plant either by direct generating growth regulators or by inhibition of pathogens (Tariq *et al.,* 2009; Raaijmakers *et al*., 2002; Korejo *et al*., 2019). The fluorescent *Pseudomonas*, which colonizes root of plants, are described to be aggressive to soilborne pathogens of plant (Siddiqui *et al*., 2000; Siddiqui & Ehteshamul-Haque, 2001; Landa *et al*., 2004; Noreen *et al*., 2018ab). Many report showed that, *Rhizobium* and *Bradyrhizobium* have potential as biocontrol agents of plant pathogens and production of certain antibiotics and siderophores (De Meyer & Hofte,1997; Noreen *et al.,* 2015; 2018b) and enhanced nitrogen fixation are considered as mechanism in improved growth and yield of plant (Rehman *et al*., 2010).

The present study explored that the various strains of fluorescent *Pseudomonas* and rhizobia significantly improved growth of chili plants. There are reports that *Rhizobium* strains colonize the roots of tomato and chili plants not only promote their growth also enhance the yield and quality fruits (Garcia-Fraile *et al*., 2012; Parveen *et al.,* 2008). Being natural inhabitant of rhizosphere and rhizoplane of crop plants, future of PGPR and rhizobia as a biocontrol agent against multiple pathogens is seems vast. PGPR and rhizobia base bio-pesticides should be develop commercially for the management of root rotting fungi and root knot nematode affecting chili.

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