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ROLE OF MUNGBEAN ROOT NODULE ASSOCIATED FLUORESCENT *PSEUDOMONAS* AND RHIZOBIA IN SUPPRESSING THE ROOT ROTTING FUNGI AND ROOT KNOT NEMATODES IN CHICKPEA (C*ICER ARIETINUM* L.)

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Abstract

Three isolates each of fluorescent *Pseudomonas* (NAFP-19, NAFP-31 and NAFP-32) and rhizobia (NFB- 103, NFB-107 and NFB-109) which were originally isolated from root nodules of mungbean (*Vigna radiata*) showed significant biocontrol activity in the screen house and under field condition, against root rotting fungi *viz*., *Macrophomina phaseolina, Fusarium solani, F. oxysporum* and *Rhizoctonia solani* evaluated on chickpea. Biocontrol potential of these isolates was also evaluated against *Meloidogyne incognita*, the root knot nematode. Application of *Pseudomonas* and rhizobial isolates as a soil drench, separately or mixed significantly reduced root rot disease under screen house and field conditions. Nematode's penetration in roots was also found significantly less in rhizobia or *Pseudomonas* treatments used separately or mixed as compared to control. Fluorescent *Pseudomonas* treated plants produced greater number of nodules per plant than control plants and about equal to rhizobia treated plants, indicating that root nodule associated fluorescent *Pseudomonas* enhance root nodulation.

Key words: Chickpea, Rhizobia, Fluorescent *Pseudomonas,* Nodulation, Root rotting fungi, Nematode.

Introduction

Rhizobia are considered scientifically and economically important microorganisms because of their ability to fix atmospheric nitrogen in leguminous plants (Hynes & O'Connel, 1990; Sprent, 2001). They have unique ability to induce nodule formation in roots of leguminous host plants by the production of specific signal molecules called Nod factors (Lerouge *et al.,* 1990; Truchet *et al.,* 1991). They convert nitrogen into ammonia for uptake by host plants while legumes provide nutrients to rhizobia (Spaink, 2000). Some isolates of rhizobia can reduce attack of soil-borne root infecting fungi, both in leguminous and non-leguminous plants along with their ability to fix nitrogen (Ehteshamul-Haque & Ghaffar, 1993; Siddiqui *et al.,* 1998a; 1998b).

Of the various rhizosphere bacteria, those belonging to fluorescent *Pseudomonas* are considered as aggressive colonizers of the rhizosphere of various crop plants and have shown significant activity against soil-borne plant pathogens (Siddiqui & Ehteshamul-Haque, 2001; Siddiqui *et al*., 2000; Weller *et al*., 2002; Whipps, 2001). The role of antifungal metabolite 2, 4-diacetylphyloglucinol, from species of fluorescent *Pseudomonas* in root disease suppression was reported by Raaijmakers & Weller (1998). Weller (1988) described the active mechanism for the control of root infecting fungi by the production of antibiotics and the competition for iron by the release of siderophores. Biocontrol potential of fluorescent *Pseudomonas* associated with the rhizosphere, rhizoplane (Siddiqui *et al.,* 2000; Ehteshamul-Haque *et al.,* 2007a), endo-root (Afzal *et al.,* 2013; Tariq *et al.,* 2009; 2014; Shafique *et al.,* 2015) and mycorrhizosphere (Bokhari *et al.,* 2014) is well documented. However, the biocontrol role of fluorescent *Pseudomonas* associated with root nodules received less attention (Batool *et al.,* 2013; Issar *et al.,* 2012). According to previous studies, it was thought that the root nodules of leguminous plants only contain rhizobia. But recent research studies provided some indication that besides rhizobia, root nodules also contain other bacteria (Pandya *et al.,* 2013). Present report describes the biocontrol potential of some isolates of fluorescent *Pseudomonas* and rhizobia originally isolated from root nodules of mungbean (*Vigna radiata*) against root rotting fungi and root knot nematode on chickpea (*Cicer arietinum* L.).

Materials and Methods

Cultures of fluorescent *Pseudomonas* and rhizobia: Cultures of fluorescent *Pseudomonas* (NAFP-19, NAFP-31 and NAFP-32) and rhizobia (NFB-103, NFB-107 and NFB-109) used in this study were originally isolated from root nodules of mungbean by Noreen *et al.* (2015). These isolates have shown promising biocontrol activity both *in vitro* and *in vivo* against root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* on mungbean. Their isolation method and identification has been reported elsewhere (Noreen *et al*., 2015).

Screen house experiment: Non- sterilized, naturally infested, sandy loam soil, pH 8.0, with a moisture holding capacity of 40% was obtained from the field of Department of Botany, University of Karachi and transferred into 21 cm diameter earthen pots at 1 kg per pot. The soil was found to be naturally infested by 3-6 sclerotia of *Macrophomina phaseolina* g^{-1} of soil as determined by wet sieving and dilution technique (Sheikh & Ghaffar, 1975); 5-10% colonization by *Rhizoctonia solani* on sorghum seeds when used as baits (Wilhelm, 1955), and 3000 cfu.g^{-1} of soil of mixed population of *Fusarium solani* and *F. oxysporum* as determined by a soil dilution technique (Nash & Synder, 1962). Eight chickpea (*Cicer arietinum* L.) seeds were sown in each pot after applying 25mL bacterial suspension of NAFP-19 (3.3 x 10^8 cfu/mL), NAFP-32 (2.5 x 10^8 cfu/mL), NAFP-31 (1.4 x 10^8 cfu/mL), NFB-103 (3.6 x 10^8 cfu/mL), NFB-107 (3.4 x 10^8 cfu/mL), and NFB-109 (4.7 x 10^8 cfu/mL) separately or mixed. After germination four seedlings were kept in each pot and excess were removed. Each pot was then inoculated with 1000 eggs/juveniles of *Meloidogyne incognita*, the root knot nematode. The pots with untreated soil served as control. While carbendazim (200 ppm) at 25 mL per pot served as positive control against root infecting fungi and carbofuran at 0.5 g per pot as positive control against nematode. The experiment was conducted in complete randomized block design with four replicates.

After 45 days, heights and fresh weights of roots and shoots of all plants were recorded. To determine the infection by root-infecting fungi, roots of tested plants were washed in running tap water, surface sterilized in 1% $Ca(OCl)_2$ and, 1cm long 5 root pieces were inoculated on PDA Petri plates containing penicillin (100,000 units/L) and streptomycin sulphate (0.2g/L). The plates were incubated at room temperature and the infection was calculated as follows:

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

Nematode's population inside the root was estimated as described by Siddiqui *et al.* (2000).

Field plot experiments: These experiments were conducted at Crop Diseases Research Institute (CDRI) of Pakistan Agricultural Research Council (PARC), Karachi University Campus, Karachi, in plots of 2x2 meters in complete randomized block design each with four replicates. The field soil had a natural infestation of *Macrophomina phaseolina*,5-16 sclerotia/g of soil, 5-12% colonization of *Rhizoctonia solani* on sorghum seeds used as baits and 2500 cfu/g of soil of mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution technique. Twelve chickpea (*Cicer arietinum* L.) seeds were sown in each row. Cell suspensions of fluorescent *Pseudomonas* NAFP-19 (2.3x 10^8 cfu/mL), NAFP-31 (3.5 x 10^8 cfu/mL), NAFP-32 (3.2 x 10^8 cfu/mL) and rhizobia NFB-103 (4.7 x 10^8 cfu/mL), NFB-107 (3.4 x 10^8 cfu/mL) and NFB-109 (3.8 x 10^8 cfu/mL) were drench separately in planting rows @ 100 mL/meter. In another set of experiment mixed suspension of rhizobia and fluorescent *Pseudomonas* were applied. After germination each row was inoculated with aqueous suspension of *M. incognita* at 1000 eggs/juveniles per meter row. Carbendazim (200 ppm) at 200 mL/meter row served as positive control against root rotting fungi, whereas, carbofuran 1 g per meter served as positive control against nematode. Each treatment was replicated four times and regularly watered after 2-3 days of interval. To determine the efficacy of fluorescent *Pseudomonas* and rhizobia on the root infecting fungi and plant growth, plants were uprooted (4 plants from each replicate) after 45 and 90 days of sowing. Infection by root infecting fungi was determined as mentioned above. Data on plant growth and root nodulation were also recorded. Data was statistically analyzed using analysis of variance (ANOVA) and Least Significant Difference (LSD) was also calculated to compare the treatments (Gomez &Gomez, 1984).

Results

Screen house experiment: Combined treatment of NAFP-31 and NFB-107 produced maximum plant height as compared to other treatments, while maximum shoot weight was obtained in treatment with NFB-107. Maximum root length was observed in treatment of NAFP-19 show significant effect ($p<0.05$) and maximum root weight was observed in control as compared to other treatments (Table 1). The largest number of nodules was produced by NAFP-32, NFB-107 and NFB-109 which showed significant ($p<0.05$) difference, while maximum number of fruit was produced by NFB-107. Maximum fruit weight was observed in plants treated with NAFP-32 and NFB-107. While the attack of nematode was significantly ($p<0.05$) reduced in plants treated with NAFP or rhizobia alone or combined (Table 1). Complete suppression of *R. solani* was observed in plants treated with NAFP-31, NAFP-19, NFB-103 and combined treatment of NAFP-32 with NFB-107, while in case of *M. phaseolina* maximum suppression was observed in NAFP-19, NAFP 31 and NFB-107 treatment as compared to other treatments. Maximum inhibition of *F. solani* was observed in combined treatment of NAFP-19 with NFB-103 while *F. oxysporum* was not found in NAFP-32, NAFP-19, NAFP-31, NFB-109, NFB-103 alone or combined treatment of NAFP-19 with NFB-107, NAFP-19 + NFB-109, NAFP-32 + NFB-107, NAFP-32 + NFB-103 and NAFP-31 + NFB-103 (Table2).

Field plot experiments: Combined treatment by NAFP-19 with NFB-103 resulted in the maximum plant height as compared to other treatments after 45 days of growth, while after 90 days interval combined treatment of NAFP-19 with NFB-107 caused maximum increase in height of plant (Table 3). After growth of 45 days, maximum shoot weight was obtained in combined treatment of NAFP-19 with NFB-103; maximum root length was observed in combined

treatment of NAFP-31 with NFB-103, maximum root weight was obtained by combined treatment of NAFP-19 with NFB-103 while after 90 days combined treatment of NAFP-31 with NFB-107 showed significant increase in weight of the plants, the combined treatment of NAFP-32 with NFB-109 showed maximum root length, whereas, individual treatment of NAFP-19 showed maximum root weight. Largest number of nodule were found in plants treated with combination of NAFP-32 with NFB-109 (45 days), while after 90 days individual treatment with NAFP-19 and combined treatment of NAFP-31 with NFB-107 produced larger number of nodules as compared to control (Table 3). After 45 days, maximum fruit production as well as fruit weight was observed in treatment with NFB-103, while after 90 days maximum fruit production was observed in NFB-107 as well as by the combined treatment of NAFP-19 with NFB-107, while maximum fruit weight was observed in plants treated with combination of NAFP-19 with NFB-103. Maximum inhibition of the infestation of nematode in plants was observed in combined treatment by NAFP-19 with NBF-103 as compared to control and other treatments after 45 days, after 90 days individual treatment with NAFP-31 as well as combined treatment of NAFP-19 with NFB-103 reduced the attack of nematodes (Table 3). *Fusarium solani* infection was significantly suppressed with the combined treatment of NAFP-19 with NFB-107 as compared to the control in 45 days, while after 90 days combined treatment of NAFP-19 with NFB-103 and NAFP-31 with NFB-109 showed maximum suppression (Table 4). Complete inhibition of *M. phaseolina* was observed in combined treatment of NAFP-32 with NFB-109 and NAFP-31 with NFB-109 in 45 days interval, while after the interval of 90 days individual treatment of NAFP-31 as well as combined treatment of NAFP-19 with NFB-107 and NAFP-32 with NFB-107 showed complete inhibition of *M. phaseolina* infection. Infection of *R. solani* was not observed whereNAFP-19 used alone and combined treatments of NAFP-32 with NFB-107, NAFP-32 with NFB-109, NAFP-31 with NFB-107, NAFP-31 with NFB-109 and NAFP-31 with NFB-103 after 45 days, while after 90 days NAFP-19, NAFP- 31, NFB-107 and NFB-103 as well as combined treatment of NAFP-19 with NFB-107, NAFP-19 with NFB-109, NAFP-19 with NFB-103, NAFP-32 with NFB-107, NAFP-32 with NFB-109 and NAFP-31 with NFB-107 showed complete inhibition of fungus (Table 4). While in case of *F. oxysporum*, complete inhibition was observed in individual treatment of NAFP-19, NAFP-32, NAFP-31, NFB-107, NFB-109 and NFB-103 as well as combined treatment of NAFP-19 with NFB-107, NAFP-19 with NFB-109, NAFP-19 with NFB-103, NAFP-32 with NFB-107, NAFP-32 with NFB-109, NAFP-31 with NFB-107, NAFP-31 with NFB-109 and NAFP-31 with NFB-103, while after 90 days the infection of *F. oxysporum* was completely suppressed by individual treatment of NAFP-31, NFB-107 as well as by the combined treatment of NAFP-31 with NFB-109 (Table 4).

Table 1. Effect of soil drench with different isolates of fluorescent *Pseudomonas* and rhizobia on plant growth, root nodulation and nematode infection on chickpea in screen house experiments.

Treatments	Growth Parameter							
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Number of nodules	Number of Fruits	Weight of fruits (g)	Juveniles & female/ gm root
Control	25.12	3.69	28.22	7.72	6	2	0.27	24
Carbendazim	26.62	3.69	26.68	6.66	8	2	0.34	16.5
Carbofuran	26.81	3.71	29.62	6.98	10	2	0.51	9
NAFP-19	27.14	3.48	34.02	6.6	9	2	0.48	8
NAFP-32	25.77	3.33	25.74	6.4	15	2	0.54	10.5
NAFP -31	25.35	3.18	26	5.18	14	2	0.41	8.25
NFB-107	27.43	4.00	24.43	5.01	15	3	0.54	11
NFB-109	25.63	3.32	24.39	5.81	15	2	0.48	8.25
NFB-103	24.91	3.39	26.93	6.82	14	2	0.37	10.5
NAFP-19 + NFB-107	22.5	2.92	25.65	6.94	14	2	0.43	10.25
NAFP-19 + NFB-109	22.75	2.70	19.81	4.64	10	2	0.33	7.25
NAFP-19 + NFB-103	23.75	2.97	21.25	5.39	13	2	0.49	6.75
NAFP-32 + NFB-107	23.54	2.67	25.89	5.2	11	2	0.41	11.75
NAFP-32 + NFB-109	26.93	3.63	25.37	6.24	10	2	0.38	8.25
NAFP-32 + NFB-103	26.18	3.23	24.68	4.33	10	2	0.36	9.25
NAFP-31 + NFB-107	29.37	3.73	24.87	5.45	14	2	0.45	10.25
NAFP-31 + NFB-109	27.91	3.39	25.43	4.77	10	2	0.35	11
NAFP-31 + NFB-103	24.27	3.06	24.08	4.71	8	1	0.34	8.5
$LSD_{0.05}$	**4.25[1]**	**0.98[1]**	**5.75[1]**	**2.53[1]**	**4[1]**	**0.81[1]**	**0.19[1]**	**3.64[1]**

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

Table 2. Effect of different isolates of fluorescent *Pseudomonas* and rhizobia on root infection of chickpea by *Macrophomina phaseolina, Fusarium solani, F. oxysporum* and *Rhizoctonia solani* in screen house experiments.

Treatments	Infection %			
	M. phaseolina	*R. solani*	*F. solani*	*F. oxysporum*
Control	75	37.5	100	12.5
Carbendazim	68.7	18.7	87.5	6.2
Carbofuran	81.2	31.2	68.7	6.2
NAFP-19	6.2	0	81.2	0
NAFP-32	12.5	6.2	87.5	0
NAFP -31	6.2	0	68.7	0
NFB-107	6.2	25	81.2	6.2
NFB-109	12.5	12.5	81.2	0
NFB-103	18.7	0	87.5	0
NAFP-19 + NFB-107	25	6.2	68.7	0
NAFP-19 + NFB-109	37.5	12.5	75	12.5
NAFP-19 + NFB-103	25	12.5	37.5	6.2
NAFP-32 + NFB-107	25	0	62.5	0
NAFP-32 + NFB-109	31.2	12.5	93.7	6.2
NAFP-32 + NFB-103	43.7	18.7	100	0
NAFP-31 + NFB-107	31.2	37.5	100	12.5
NAFP-31 + NFB-109	50	18.7	93.7	12.5
NAFP-31 + NFB-103	43.7	12.5	75	0
$LSD_{0.05}$	**Treatments = 13.4[1]**		**Pathogens = 6.5[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 $p< 0.05$

Discussion

Chickpea (*Cicer arietinum* L.) is an important pulse crop, is susceptible to the root-knot nematode *Meloidogyne* spp., and root infecting fungi (Ehteshamul-Haque *et al.*, 1997; Sultana *et al.*, 2000). The interaction between *M. incognita* and *M. phaseolina* causes a root-rot disease complex that severely damages this important crop (Siddiqui & Husain 1991; 1992). In the present study, 3 isolates of fluorescent *Pseudomonas* and rhizobia isolated from the root nodules of *Vigna radiate* showed significant suppressive effect on root infecting fungi such as *Macrophomina phaseolina, Fusarium solani, F. oxysporum* and *Rhizoctonia solani* and root knot nematode *M. incognita* both in screen house and field experiments. Suppression of root rot diseases by the fluorescent *Pseudomonas* associated with rhizosphere, rhizoplane and endo-root is well documented (Afzal *et al.*, 2013; Ehteshamul-Haque *et al.*, 2007ab; Haas & Defago, 2005; Tariq *et al.*, 2009, 2014; Weller *et al.*, 2002). The present study indicates that root nodule associated fluorescent *Pseudomonas* also has great potential against soil-borne plant pathogens.

Active fixation of nitrogen by legume-rhizobia symbiosis is critical to agricultural crop production (Smith &Hume, 1987; Vance, 1997; Pepper, 2000). Rhizobia have also been reported to suppress soil-borne plant pathogens both on leguminous and non-leguminous plants (Ehteshamul-Haque & Ghaffar, 1993; Ehteshamul-Haque *et al.*, 2007a). Co-inoculation of rhizobia along with plant growth promoting bacteria (PGPB) can be used to increase crop production. In this study co-inoculation of rhizobia and PGPR significantly increased the growth of chickpea as well as reduced the infection caused by plant parasitic pathogen in comparison to individual treatment of rhizobia and *Pseudomonas*. Benefits with co-inoculation of *Rhizobium* or *Bradyrhizobium* species with PGPB has been reported (Li & Alexander, 1988; Srinivasan *et al.*, 1996; Zhang *et al.*, 1996; Bullied *et al.*, 2002). Increase in plant growth can occur directly or indirectly by rhizobacteria. Indirect mechanism involves the production of metabolites such as antibiotics, siderophore or HCN that decreases the growth of phytopathogens and other deleterious microorganism. Direct mechanism involves the production of plant growth hormones, or improvement in plant nutrient uptake (Klopper, 1993; Glick *et al.*, 1995). The increase in root nodulation and nitrogen fixation may occur by the production of flavonoid like compounds and stimulating the legume to produce more flavonoid signal molecule by rhizobacterial strains (Parmar & Dadarwal, 1999).

In the present study the nodule associated *Pseudomonas* significantly increased the plant height, plant weight and produced better root growth. The plants showing increased root development specifies increased nutrient uptake by the plant and some PGPB are known to increase the root growth by the production of indole-3-acetic acid (Barbiei & Galli, 1993; Srinivasan *et al.*, 1996).There are reports that mixed application of bradyrhizobia and *P. aeruginosa* increase root nodulation in chickpea (Izhar *et al.*, 1995) and also improved nitrogen fixation in soybean (Dashti *et al.*, 1995; Cheboter *et al.*, 2001). During the experiments it was observed that fluorescent *Pseudomonas* also inhabited root nodule along with rhizobia and they possess biocontrol potential against root infecting fungi and root knot nematode. This may help in development of a valuable crop management tool against soil-borne root infecting fungi and parasitic nematodes. However, their role in root nodulation needs further investigation.

Table 3. Effect of different isolates of *Pseudomonas* and rhizobia on the growth, root nodulation and nematode infection of chickpea under field condition.

Treatments	Growth parameter															
	Shoot length (cm)		Shoot weight (g)		Root length (cm)		Root weight (g)		Number of nodules		Number of fruits/plant		Weight of fruits/plant (g)		Juveniles & female/ g root	
Days	**45**	**90**	**45**	**90**	**45**	**90**	**45**	**90**	**45**	**90**	**45**	**90**	**45**	**90**	**45**	**90**
Control	27.87	29.81	5.59	6.51	11.02	11.43	1.15	1.12	0	0	1.91	4.62	1.39	2.32	32.5	34
Carbendazim	28.31	32.68	6.46	7.95	14.43	13.31	1.21	1.70	1.25	1.25	6.14	8.68	1.07	3.12	31.5	32.5
Carbofuran	28.62	33.59	6.23	7.81	14.76	13.67	1.27	1.78	1.26	1.5	6.23	9.67	1.12	4.23	18.5	17.61
NAFP-19	29.31	31.18	6.6	6.98	12.62	13.93	1.58	2.37	4.87	11.25	4.20	7.68	1.1	3.73	14.25	26
NAFP-32	26.33	30.5	5.65	6.46	13.25	11.10	1.43	1.38	5.83	6.25	2.58	5.87	0.96	3.72	12.5	21.5
NAFP -31	27.68	30.93	6.63	10.15	13.75	14.5	1.20	1.70	3.75	1.25	3.64	6	0.55	3.06	12	12.75
NFB-107	26.91	34.23	5.9	8.56	11.39	13.85	1.18	1.81	7.5	8.75	3.93	6.68	0.72	3.37	12.75	16
NFB-109	29	30.23	5.83	6.58	10.68	12.04	0.99	1.50	5.75	7.75	4.06	7.16	1.13	4.26	17	18.75
NFB-103	26.85	30	7.31	10.75	12.41	14.83	1.06	1.79	3.75	6.25	7.37	12.06	4.74	6.36	17	19.5
NAFP-19 + NFB-107	27.39	35.9	7.09	9.50	13.56	12.4	1.65	1.64	5	6.25	3.25	6.68	1.28	4.63	14	16.5
NAFP-19 + NFB-109	25.56	27.87	5.07	6.54	14.25	11.78	1.71	1.28	5	7.5	2.66	5.65	1.03	3.78	13.75	15.75
NAFP-19 + NFB-103	29.74	31.67	8.79	6.8	13.64	13.76	1.73	2.01	3.75	7.5	6.81	13.67	1.02	7.86	10	12.75
NAFP-32 + NFB-107	25.11	30.71	5.87	8.39	13.58	13.20	1.26	1.63	6.25	10	3.83	7.52	0.66	4.28	12	13.75
NAFP-32 + NFB-109	27.06	28.27	5.77	6.78	12.03	15.08	1.27	1.71	8.75	9.5	2.27	6.37	0.58	2.11	18	23
NAFP-32 + NFB-103	25.93	31.84	5.93	7.66	13.31	12.68	1.27	1.44	5.75	10	3.66	6	0.73	3.16	15	17.5
NAFP-31 + NFB-107	27.72	35.55	6.48	10.88	14.68	12.43	1.59	1.92	7.5	11.25	2.12	5.87	4.39	5.03	17.25	24
NAFP-31 + NFB-109	26.55	26.17	6.72	6.63	13.42	13.09	1.06	1.00	2.5	4.5	1.68	3	2.40	3.32	16.25	21.5
NAFP-31 + NFB-103	25.95	28.75	6.15	6.81	15.35	13.43	1.43	1.23	6.25	8.5	2.06	4.41	3.89	4.41	15.5	14.25
$LSD_{0.05}$	**ns**	**ns**	**ns**	**ns**	**2.94[1]**	**3.49[1]**	**ns**	**1.37[1]**	**8.77[1]**	**11.8[1]**	**5.01[1]**	**6.25[1]**	**2.00[1]**	**3.79[1]**	**3.99[1]**	**4.88[1]**

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

Table 4. Effect of different isolates of *Pseudomonas* and rhizobia on root infection by *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* on chickpea roots under field conditions.

Treatments	Infection %							
	M. phaseolina		*R. solani*		*F. solani*		*F. oxysporum*	
Days	**45**	**90**	**45**	**90**	**45**	**90**	**45**	**90**
Control	43.7	43.7	6.2	18.7	100	100	25	62.5
Carbendazim	31.2	37.5	6.2	12.5	100	100	12.5	25
Carbofuran	37.5	62.5	6.2	12.5	91.7	100	6.2	31.2
NAFP-19	31.2	25	0	0	75	100	0	31.2
NAFP-32	37.5	18.7	6.2	6.2	87.5	100	0	18.7
NAFP -31	18.7	0	12.5	0	93.7	93.7	0	0
NFB-107	12.5	6.2	6.2	0	93.7	75	0	0
NFB-109	18.7	18.7	6.2	12.5	75	75	0	6.2
NFB-103	25	6.2	6.2	0	87.5	67.7	0	6.2
NAFP-19 + NFB-107	18.7	0	6.2	0	62.5	75	0	18.7
NAFP-19 + NFB-109	31.2	12.5	6.2	0	75	75	0	18.7
NAFP-19 + NFB-103	25	6.2	12.5	0	75	62.5	0	25
NAFP-32 + NFB-107	25	0	0	0	81.5	93.7	0	12.5
NAFP-32 + NFB-109	0	6.2	0	0	93.7	87.5	0	12.5
NAFP-32 + NFB-103	25	37.5	6.2	12.5	100	93.7	6.2	37.5
NAFP-31 + NFB-107	12.5	18.7	0	0	81.2	75	0	25
NAFP-31 + NFB-109	0	6.2	0	6.2	75	62.5	0	0
NAFP-31 + NFB-103	25	37.5	0	6.2	68.7	93.7	0	6.2
$LSD_{0.05}$	**Treatments = 10.46[1]**		**Pathogens = 5.07[2]**		**Time = 3.58[3]**			

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

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

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

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