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Field evaluation of two bacterial antagonists, *Pseudomonas putida* (PDBCAB 19) and *P. fluorescens* (PDBCAB 2) against wilt and root- rot of chickpea

R. RANGESHWARAN, R. D. PRASAD and C. P. ANUROOP
Project Directorate of Biological Control (ICAR)
Post Bag No.2491, H. A. Farm Post, Bellary Road
Bangalore 560 024, Karnataka, India
E-mail: pdblc@kar.nic.in

ABSTRACT: **Talc based formulations of two antagonistic bacteria *viz.*, *Pseudomonas putida* (PDBCAB 19) and *P. fluorescens* (PDBCAB 2) were evaluated against natural incidences of wilt and wet root-rot of chickpea. Observations on rhizoctonia root-rot incidence indicated that the disease was prevalent up to 60 days of plant growth whereas fusarial wilt was observed from 60 days. At 30th day, highest root-rot incidence (10.8%) was observed in pathogen control plots and minimum was in fungicide treated plots (3.1%). *P. fluorescens* (PDBCAB 2) treated plots also exhibited low root-rot (4.4%). However, at day 60 lowest root-rot incidence (5%) was recorded in *P. fluorescens* (PDBCAB 2) treated plots and highest root-rot incidence (13.9%) was observed in pathogen control. Low root-rot incidence (5.8%) was also noticed in *P. putida* (PDBCAB 19) treated plots. *Fusarium* wilt was lowest (3.3%) in *P. putida* (PDBCAB 19) treated and highest (13.3%) in control plots at day 90. Combination of both *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) exhibited suppression of root-rot and wilt incidences by 5.8 and 7.5 per cent, respectively. Highest plant stand and seed yield was observed in *P. fluorescens* (PDBCAB 2) treated plots. There were no significant differences in the shoot and root lengths among bioagent treatments, however, vigour index was highest in plots treated with a combination of *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19).**

KEY WORDS: Antagonistic bacteria, chickpea, root-rot, wilt

Wilt caused by *Fusarium oxysporum* f. sp. *ciceri* and root-rot by *Rhizoctonia solani* Kuhn in chickpea is very serious. A rough estimate indicates that losses due to the wilt complex may be around 10 to 15 per cent each year. In severe epidemics, crop losses may go as high as 60-70 per cent. Damage is up to 61 per cent at seedling stage and 43 per cent at flowering stage (Haware and Nene, 1980).

The disease suppressing and beneficial effects of rhizosphere colonizing bacteria have been well studied (Ryder *et al.*, 1994; Vidhyasekaran, 1998; Rangeshwaran and Prasad, 2000a and 2000b). Seeking alternatives to the use of chemical fungicides is now the aim of any biocontrol strategy. Most of the biocontrol work using bacterial antagonists has been confined to lab or greenhouse studies (Vidhyasekaran, 1998). We had already established the beneficial effects of two rhizosphere bacteria *viz.*, *Pseudomonas fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19), selected out of three hundred rhizosphere isolates (Rangeshwaran and Prasad, 2000a; 2000b). The two antagonists were able to inhibit *F. oxysporum* f. sp. *ciceri* and *R. solani* in dual

culture and also under greenhouse conditions. They also exhibited moderate root colonozing and growth promoting abilities (Rangeshwaran and Prasad, 2000b).

To further evaluate the performance of the two rhizosphere isolates, an experiment was conducted under field conditions to test the inhibitory potential of the antagonists against natural incidences of wilt (*Fusarium oxysporum*) and root-rot (*Rhizoctonia solani*) in chickpea.

MATERIALS AND METHODS

Bacterial antagonists

The two antagonistic rhizosphere bacteria viz., *Pseudomonas fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) were selected from the culture collection of Project Directorate of Biological Control (PDBC), Bangalore. The two antagonists were chosen from three hundred rhizosphere isolates and their biocontrol ability has already been established against chickpea wilt pathogens (Rangeshwaran and Prasad, 2000b).

Formulation

Talc based formulations of selected antagonists were prepared as described by Vidhyasekeran *et al.* (1996). The pH of the formulation was adjusted to 7.0 using calcium carbonate. Ten grams of carboxymethyl cellulose (CMC) per kg of carrier was used as adhesive. Selected rhizosphere bacteria were grown in nutrient broth (NA) for 48 to 72h in a rotary shaker to obtain a minimum population of 9×10^8 colony forming units (CFUs)/ml. The suspensions were mixed with sterile carrier (400ml/kg) and air-dried. Chickpea seeds (Annegiri) were wetted with CMC (1%) and uniformly coated with the respective formulations (5 gm/kg seed) before sowing.

Field Trial

A plot used regularly for growing chickpea was selected at the University of Agricultural Sciences, Bangalore for screening the two selected bacterial antagonists. The treatments included *P. fluorescens* (PDBCAB 2) at 5g/kg, *P. putida* (PDBCAB 19) at 5g/kg, combination of both *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) at 10g/kg, fungicide Captan at 2g/kg and untreated pathogen control. Individual plots measured 3x3m in a randomized block design with three replications. Sowing was done with a row spacing of 25cm and spacing between plants was 15cm. Observations on seedling emergence, root-rot and wilt incidences, plant stand, pod yield (kg/ha), shoot/root length and vigour index were recorded after 45 days. The results were analyzed as per analysis of variance. Vigour index was calculated by multiplying the per cent seedling emergence with the sum of root and shoot length.

RESULTS AND DISCUSSION

The effect of seed treatment with talc based formulations of the two antagonistic bacteria *viz.*, *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) was tested against natural incidences of wilt (*Fusarium* f. sp. *ciceri*) and root-rot (*R. solani*) infecting chickpea. Significant differences were obtained in control of *Rhizoctonia* root-rot. As indicated in Table 1, at 30th day, the lowest root-rot incidence (3.1%) was in the fungicide treatment and the highest (10.8%) was observed in pathogen control. The root-rot incidence ranged between 4.4 and 6.9 per cent in antagonists treated plots. On the 60th day the lowest disease incidence (5%) was observed in *P. fluorescens* (PDBCAB 2) treated plots and again highest (13.9%) was in pathogen check. Low root-rot incidence (5.8%) was also observed in *P. putida* (PDBCAB 19) treated plots. On the 90th day no significant root-rot incidences were observed in all the treatments. Ishrat-Izar *et al.* (1995) reported that the growth promoting bacterium *P. aeruginosa* (strains Pa6 and Pa12) significantly reduced infection by *Macrophomina phaseolina* and *R. solani* on chickpeas. Effective control of rice sheath blight pathogen *R. solani* was obtained when talc based powder formulation of *P. fluorescens* strain Pf-1 was applied to seed, foliage, root and soil (Vidhyasekaran and Muthamilan, 1999).

Table 1. Effect of antagonists on and *Rhizoctonia* root rot incidence in chickpea

Treatment/dosage	Plants exhibiting root rot (%) days after		
	30	60	90
Pseudomonas fluorescens (PDBCAB 2) seed treated at 5g/kg	4.4 (12.1)	5.0 (12.8)	0.8 (4.2)
P. putida (PDBCAB 19) seed treated at 5g/kg	6.9 (15.2)	5.8 (13.9)	0.5 (3.4)
P. fluorescens + *P. putida* seed treatment at 10g/kg	5.8 (13.9)	7.5 (15.9)	1.1 (5.0)
Fungicide	3.1 (9.9)	9.5 (17.8)	1.4 (6.7)
Pathogen control	10.8 (19.2)	13.9 (21.9)	1.4 (6.7)
CD ($p< 0.05$)	(3.43)	(3.90)	NS

NS = not significant *

Figures in parentheses are angular transformed values.

Fusarium wilt was observed on the 60th day and minimum wilted plants (1.7%) were observed in *P. fluorescens* (PDBCAB 2) treated plots and the highest (8.3%) in pathogen control (Table 2). In other treatments, the per cent wilted plants ranged between 2.2 and 3.0. However, on the 90th day minimum (3.3%) wilted plants were observed in the plots seed treated with *P. putida* (PDBCAB 19) and again the highest (13.3%) in pathogen control. Per cent wilted plants in fungicide treatment were 4.2 and in seed treatment with the antagonists, the per cent wilted plants ranged between 5.8 and 5.0.

Table 2. Effect of antagonists on *Fusarium* wilt incidence in chickpea

Treatment/dosage	Plants exhibiting root rot (%) days after		
	30	60	90
Pseudomonas fluorescens (PDBCAB 2) seed treated at 5g/kg	0.0	1.7 *(7.2)	5.0(12.8)
P. putida (PDBCAB 19) seed treated at 5 g/kg	0.0	2.2(8.5)	3.3(10.5)
P. fluorescens + *P. putida* seed treatment at 10g/kg	0.0	3.0(10.0)	5.8(13.9)
Fungicide	0.0	2.5(9.0)	4.2(12.4)
Pathogen control	0.0	8.3(9.5)	13.3(21.4)
CD ($p<0.05$)	-	NS	3.33

NS = not significant *

Figures in parentheses are angular transformed values.

Seed bacterization with fluorescent pseudomonads isolated from rhizosphere of crop plants reduced the number of wilted chickpea plants in wilt sick soil (*F. oxysporum* f. sp. *ciceri*) (Kumar, 1998). Gupta *et al.* (1996) reported that colonization of chickpea roots by herbicide tolerant strains of *P. fluorescens* and *P. putida* significantly reduced wilt disease caused by *F. oxysporum* f. sp. *ciceri*.

Observations on plant stand and yield of chickpea were also undertaken after 90 days (Table 4). Maximum plant stand (83.1%) was observed in plots treated with *P. fluorescens* (PDBCAB 2) and minimum (54.1%) in pathogen control plots. *P. putida* (PDBCAB 19) treated plots also gave good plant stand (81.1%). Highest pod yield of 911.7kg/ha was observed in plots with *P. fluorescens* (PDBCAB2) seed treatment and the lowest (539 Kg/ha) was in pathogen control plots. High yield (888.3 kg/ha) was also obtained in *P. putida* (PDBCAB 19) treated plants. In other treatments the yield ranged between 801.8 and 837.9kg/ha. Per cent seedling emergence was the highest (85.8%) in plots seed treated with a combination of *P. fluorescens* and *P. putida* at 10g/kg and the lowest emergence (70.8%) in pathogen

Table 3. Effect of antagonists on growth of chickpea after 45 days

Treatment/dosage	Seedling emergence (%)	Shoot length (cm)	Root length (cm)	Vigour index
Pseudomonas fluorescens (PDBCAB 2) seed treated at 5g/kg	84.7 *(67.5)	30.2	13.2	3666.2
P. putida (PDBCAB 19) seed treated at 5 g/kg	84.1 (66.8)	30.4	13.7	3700.1
P. fluorescens + *P. putida* seed treatment at 10g/kg	85.8 (68.2)	31.1	12.9	3784.6
Fungicide	81.9 (65.3)	32.1	12.9	3686.0
Pathogen control	70.8 (57.4)	27.3	11.1	2706.2
CD ($p < 0.05$)	NS	NS	NS	643.18

NS = not significant *
Figures in parentheses are angular transformed values.

Table 4. Effect of antagonists on plant stand and yield of chickpea after 90 days

Treatment/dosage	Plant stand (%)	Yield (kg/ha)
Pseudomonas fluorescens (PDBCAB 2) seed treated at 5g/kg	83.1 *(65.8)	911.7
P. putida (PDBCAB 19) seed treated at 5g/kg	81.1 (64.3)	888.3
P. fluorescens + *P. putida* seed treatment at 10 g/kg	76.7 (61.1)	837.9
Fungicide	78.8 (62.6)	801.8
Pathogen control	54.1 (47.3)	539.0
CD ($p< 0.05$)	2.74	116.16

NS = not significant *
Figures in parentheses are angular transformed values.

control plots, but the treatments were not significantly different (Table 3). Surprisingly observations on growth parameters also indicated no significant differences in shoot or root length between the treatments (Table 3). Highest shoot length of 32.1 cm was observed in fungicide treated and highest root length of 13.7 cm was observed in plants seed treated with *P. fluorescens* (PDBCAB 2). However, highest vigour index of 3784.6 was derived from plots that received a combination of *P. fluorescens* and *P. putida* at 10 g/kg and lowest vigour index of 2706.2 was in pathogen check. Specific strains of *P. fluorescens* or *P. putida* group have been used as seed inoculants in crop plants to promote growth and increase yield (Kloepper *et al.*, 1980). Nautiyal (1997) reported that chickpea seed bacterization with *P. fluorescens* NBRI1303 increased the germination of seedlings by 25 per cent and reduced the number of wilted plants by 45 per cent. He also recorded increased shoot and root length ranging from 16 to 18 per cent and enhanced grain yield by 22.61 per cent. The bacterium was selected from 386 rhizosphere isolates.

In our study, the two rhizosphere antagonists *viz.*, *P. fluorescens* (PDBCAB 2) and *P. putida* (PDBCAB 19) were selected from 300 rhizosphere isolates. Their root colonizing, growth promoting and biological control ability have already been established (Rangeshwaran and Prasad, 2000a; 2000b). We emphasize that one has to select a biological control agent not merely based on its inhibitory ability but also on its root colonizing and growth promoting abilities. We have currently initiated studies on large-scale field evaluation of these two antagonists.

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