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Biological Control of *Fusarium oxysporum* f. sp. *ciceri* by Nonpathogenic *Fusarium* and Fluorescent *Pseudomonas*

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Abstract: Biocontrol efficacy of selected isolates of nonpathogenic *Fusarium* and fluorescent *Pseudomonas* was determined in growth chambers and in microplots. The selected isolates of nonpathogenic *Fusarium* and fluorescent *Pseudomonas* were evaluated singly as well as in combination against *Fusarium oxysporum* f. sp. *ciceri* (Foc) causing chickpea wilt. Under growth chambers conditions, antagonists were applied as seed and soil treatment while in microplots only seed treatment was done. Pathogenic isolate of *F. oxysporum* f. sp. *ciceri* was mixed in soil as soil treatment in all the combinations of antagonists. Under growth chamber conditions, there were 15-30 and 0-15% plants showing wilting after 30 days of sowing with treatment of nonpathogenic *Fusarium* and fluorescent *Pseudomonas*, respectively. Gradually the percentage of wilting increased and after 60 days of sowing it was 20-40 and 40-50%, respectively. The combination of Fo52 with C7R12 was the best where none of plants was observed wilted after 30 days of sowing and only 10% plants showed wilting after 60 days. Similar results were obtained from the microplot studies. The seed treatment with Fo52 and C7R12 in combination showed maximum seed germination and disease inhibition as compared to other treatment.

Key words: Biocontrol, nonpathogenic *Fusarium*, chickpea wilt, antagonism, rhizobacteria, antagonists

INTRODUCTION

The chickpea is one of the most important pulse crop grown through out India. Area and production are decreasing in Punjab due to uncertainty and risk involved in its cultivation due to occurrence of several diseases. Chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is one of the limiting factors in its cultivation. Traditionally, the use of toxic chemicals was the common tool for reducing the losses due to plant diseases. However, in recent years concern has increased regarding hazardous effects of chemicals on the life of man and animals. The intensive use of chemicals has also lead to the increase in pathogen resistance to these chemicals. Such problems have instigated the search for the alternative approaches like biocontrol in the management of diseases.

Among the biocontrol agents, the nonpathogenic isolates of *F. oxysporum* and fluorescent *Pseudomonas* are common soil inhabitants and have been widely used as antagonists against fusarial wilt of several agricultural important crops (Alabouvette *et al*., 1998; Alabouvette and Steinberg, 1995; Duijff *et al*., 1998; Fuch *et al*., 1997, 1999; Paul *et al*., 1999).

In preliminary studies the selected isolate Fo52 of nonpathogenic *F. oxysporum* and isolate Pf5 of fluorescent *Pseudomonas* isolated from rhizosphere soils of different chickpea growing area in Punjab state of India, has a potential to suppress chickpea wilt under *in vitro* conditions (Paul *et al*., 1999; Kaur, 2003). The objective of the present study was to determine the biocontrol efficacy of these selected Indian isolates and three other antagonistic isolates from France against chickpea wilt in growth chambers and in microplots when applied singly as well as in combination.

MATERIALS AND METHODS

Isolations of *F. oxysporum* and fluorescent *Pseudomonas*: The isolates of *F. oxysporum* and fluorescent *Pseudomonas* were collected from the rhizosphere soil of chickpea plant growing in different

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area in Punjab state of India. Komada's medium (Komada, 1975) was used as an agar-based selective medium to isolate *F. oxysporum* from soil samples. The isolates of fluorescent *Pseudomonas* were isolated on King' B medium (King *et al*., 1954) and purified by streaking.

Selection of pathogenic and nonpathogenic isolates of *F. oxysporum*: All the isolates of *F. oxysporum* were screened on three weeks old seedlings of chickpea for their pathogenicity test using rapid test technique (Roberts and Kraft, 1971). Isolates showing maximum wilting with in shortest duration of time were recognized as potential pathogenic, while those showing no wilting was selected as nonpathogenic. Finally the isolate Fo52 was selected as nonpathogenic and one isolate *F. oxysporum* f. sp. *ciceri* (Foc) was identified as pathogenic isolate (Kaur *et al*., 2003).

Selection of antagonistic isolates of fluorescent *Pseudomonas*: *In vitro* evaluation of fluorescent *Pseudomonas* isolates was conducted using dual culture technique on PDA with Foc (Kaur *et al*., 2003). The bacterial isolates showing maximum zone of inhibition was selected for further studies.

The isolates Fo47 and Fo47b10 of nonpathogenic *Fusarium* and C7R12 of fluorescent *Pseudomonas* were procured from INRA, Dijon France, which were used as biocontrol agents against wilt pathogens in the France. These were isolated from *Fusarium* suppressive soil from Chateaurenard region of France (Alabouvette, 1986).

Evaluation of biocontrol efficacy of antagonists: To evaluate biocontrol efficacy of antagonist, soil and seed inoculations were done to conduct bioassay in growth chambers and only seed inoculation was done for microplot studies.

Bioassay in growth chambers

Soil inoculation: The soil was sterilized twice at 120°C in the autoclave for 30 min at 24 h interval. Each pot was filled up with l00 g-sterilized soils. The pots were inoculated with the wilt pathogen, Foc at the inoculum concentration of 1×10^3 cfu g^{-1} soil. For soil inoculation of the biocontrol agents, the charcoal based formulation of the nonpathogenic *Fusarium* Fo47, Fo47b10 and Fo52 at the rate of 1×10^6 cfu g^{-1} soil and of the fluorescent *Pseudomonas* C7R12 and Pf5 at the rate of 1×10^8 cfu g^{-1} soil were added separately two days after inoculation of the pathogen. Further, two days later the surface disinfected 5 seeds of wilt susceptible variety JG-62 were planted in pots. All the treatments were replicated five times. A constant temperature of 28°C was maintained for duration of study. The irrigation was given as required to maintain optimum moisture level. The wilt incidence was recorded at regular intervals.

Seed inoculation: For seed application of the biocontrol agents, the surface sterilized seeds were treated with charcoal based formulation (Kaur *et al*., 2003) of fungal and bacterial antagonists containing the inoculum at the rate of 1×10^6 and 1×10^8 cfu g^{-1}, respectively. The carboxy methyl cellulose (CMC) was used as a sticker at the rate of 1% in the formulation. In case of combined treatments of nonpathogenic *Fusarium* and fluorescent *Pseudomonas*, the seeds were first treated with fluorescent *Pseudomonas* inoculum under suspensions at the rate of 1×10^8 cfu mL^{-1} and 24 h later seeds were inoculated with charcoal based formulation of fungal antagonists. After the treatments, the seeds were planted in pots containing 100 g soil and were inoculated with Foc at the rate of 1×10^3 cfu g^{-1} soil. The sowing was done as described for soil treatments. All the treatments were replicated five times. The irrigation was given as per requirements. The wilt incidence was recorded at regular intervals.

Bioassay in microplots: The microplots each of size 2×1.5 m size were prepared in experimental area of Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The 60 mL of Foc inoculum at 1×10^6 cfu mL^{-1} were inoculated in each plot 5 days of sowing. The chickpea seeds treated with charcoal based formulation of three nonpathogenic *Fusarium* and two fluorescent *Pseudomonas* isolates were sown after five days of inoculation of pathogen. Microplots consisted of 5 rows. Initially 25 seeds were sown in each row, however after germination only 15 seedlings were maintained for further observations. All the treatments were replicated three times.

Statistical analysis: Data from wilt incidence was subjected to ANOVA using SAS (SAS Institute Inc.) and treatments means were separated by Tukey's HSD test at $p = 0.05$. Before analysis the data was transformed by $\sqrt{x+1}$ transformation to equalize the error variances prior to analysis of variance. Untransformed data has been shown in the tables.

RESULTS AND DISCUSSION

The treatments of nonpathogenic *Fusarium* and fluorescent *Pseudomonas* singly as well in combination were highly effective to reduce the wilting in chickpea (Table 1). Only 15-30 and 20-35% plants showed wilting

after 30 and 60 days of sowing respectively when isolates of nonpathogenic *Fusarium* were applied singly as soil treatment, where as in control 100% plants were wilted after 30 days. Soil treatment with isolate C7R12 of fluorescent *Pseudomonas* protected plants from wilting up to 30 days but after 60 days of sowing 40% plants were wilted. Similarly the soil treatment with isolate Pf5 produced 15 and 50% wilted plants after 30 and 60 days of sowing. Soil treatment with combination of non-pathogenic *Fusarium* with fluorescent *Pseudomonas* gave better protection against *F. oxysporum* f. sp. *ciceri*. The combination of Fo52+C7R12 was the best in controlling *F. oxysporum* f. sp. *ciceri* infection, as there was no wilting after 30 days of sowing and only 10% wilting recorded after 60 days of sowing.

All the 5 antagonists when applied as seed treatment singly or in combination gave significant protection against wilt pathogen (Table 1). There were 15-20 and 25% plants wilted after 45 days of sowing in seed treatments in nonpathogenic *Fusarium* and fluorescent *Pseudomonas* respectively as compared to 95% wilting in control.

Germination of seeds and wilting of chickpea plant reduced significantly due to application of nonpathogenic *Fusarium* and fluorescent *Pseudomonas* singly or in combination at various stage of plant growth in microplot studies (Table 2). The 68-69, 64-65 and 68-74% seed germination was recorded due to treatments of nonpathogenic *Fusarium*, fluorescent *Pseudomonas* and combination of both antagonists respectively as compared to 57% germination in control. The combined treatment of Fo52+C7R12 gave maximum, 74% germination of seeds. Among the nonpathogenic *Fusarium* isolates, the treatment with Fo47b10 gave complete protection up to 30 day of sowing. However, 8 and 4 wilted plants were observed in plots treated with Fo47 and Fo47b10, respectively. The treatment of fluorescent *Pseudomonas* also gave better protection and only 38 and 58% plants showed wilt symptoms when treated with Pf5 and C7R12, respectively. The combination of *Fusarium* and *Pseudomonas* treatments was found better than the single application of either the antagonist. Only 0-4% wilting observed in all the 6 combined treatments after 30 days of sowing as compared to 10% wilting in control (Table 2).

Efficiency of nonpathogenic *Fusarium* and fluorescent *Pseudomonas* against fusarial wilt of chickpea had already been described in different parts of world (Alabouvette *et al*., 1998; Paul *et al*., 1999). Current studies were conducted to know about the possibility to use nonpathogenic *F. oxysporum* as seed and soil treatment under Indian conditions. The Indian strain of nonpathogenic *Fusarium* isolated from rhizosphere of chickpea also reduced the fusarial wilt in chickpea (Paul *et al*., 1999; Kaur, 2003). Fluorescent *Pseudomonas* strain, Pf5 was effective to reduce the incidence of several soil borne pathogen including, pathogenic *F. oxysporum* (Kaur *et al*., 2003; Sandhu, 2001). Individually the efficacy of nonpathogenic *Fusarium* and fluorescent *Pseudomonas* varied much from one host system to other. These results were very interesting since seed application is only economical way to apply biocontrol agent in open fields. These results obtained under field conditions in an artificially infested soil show that these biocontrol agents might be effective in grower's fields. Development of biological control products based on these strains needs technological research to study the best formulation to ensure success of the control.

Table 1: Effect of seed and soil inoculation of nonpathogenic *Fusarium oxysporum* and fluorescent *Pseudomonas* singly as well as in combination on the incidence of chickpea wilt in growth chambers at 26°C

	Wilt incidence (%) after		
	Soil inoculation		Seed inoculation
Treatments	30 days*	60 days	45 days
Fo47	30±5c	35±6d	20±6c
Fo47b10	15±5de	40±5c	20±6c
Fo52	15±5de	20±5e	15±4cd
C7R12	0±3e	40±3c	25±4c
Pf5	15±2de	50±5b	25±3bc
Fo47+C7R12	20±4d	40±5c	25±3bc
Fo47+Pf5	40±8b	50±5b	30±4b
Fo47b10+C7R12	40±6b	50±5b	25±4bc
Fo47b10+Pf5	25±4cd	50±5b	10±2d
Fo52+C7R12	0±4e	10±5f	15±6cd
Fo52+Pf5	0±5e	20±5e	20±1c
Control	100±2a	100±2a	95±5a

*Days after sowing, Nonpathogenic *F. oxysporum*- Fo52, Fo47, Fo47b10 Fluorescent *Pseudomonas*-Pf5 and C7R 12, Means±SEM within a column followed by different lower case letter(s) are significantly different (p = 0.05) using Tukey HSD test

Table 2: Fect of seed inoculation of nonpathogenic *Fusariumo xysporum* and fluorescent *Pseudomonas* singly as well as in combination on the incidence of chickpea wilt in field conditions

		Wilt incidence (%) after			
Treatments	Germination (%)	30 days*	60 days	100 days	140 days
Fo47	68±10d	8±2b	20±5g	43±2ab	46±2de
Fo47b10	69±10c	0±0d	23±6c	40±5ab	46±5de
Fo52	68±5de	4±1c	13±6f	30±5d	36±5f
C7R12	64±12gh	0±0d	26±6cd	46±4b	58±5b
Pf5	65±12f	0±0d	23±5d	36±5c	38±5fg
Fo47+C7R12	64±6gh	0±0d	26±6cd	43±3ab	48±4d
Fo47+Pf5	68±11de	4±0c	26±6cd	46±4b	52±8c
Fo47b10+C7R12	67±10e	4±0c	28±5c	30±4d	42±6e
Fo47b10+Pf5	71±11b	4±0c	38±5b	43±4ab	48±6c
Fo52+C7R12	74±12a	4±0c	13±8f	23±7e	33±6g
Fo52+Pf5	69±10c	0±0d	16±2e	30±7d	36±6f
Control	57±10h	10±0a	60±3a	100±0a	100±0a

*Days after sowing, Nonpathogenic *F. oxysporum*-Fo52, Fo47, Fo47b10, Fluorescent *Pseudomonas*-Pf5 and C7R12, Means within a column followed by different lower case letter(s) are significantly different (p = 0.05) using Tukey HSD test

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