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Synergism Between *Pseudomonas fluorescens* Migula and Thiram for the Control of Collar Rot of Chickpea

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Abstract

The efficacy of two bacterial strains of Pseudomonas fluorescens *Migula (CP 8-2 & CP 8-3) was tested in combination with thiram against collar rot (*Sclerotium rolfsii *Sacc.) of chickpea both in greenhouse and field experiments. The bacterial strains in combination with thiram gave significant reduction in collar rot incidence in both the experiments. Greenhouse studies indicated that the combination treatment of CP 8-3 with thiram was found superior in minimizing the collar rot incidence (57.6 % and 64.8 % in 1999 and 2000, respectively) in both the seasons when compared to control. In the field experiments also the combination treatments proved superior in recording low disease incidence, which ranged from 7.3 to 15.5% in Annigeri compared to 25.2 % in control. In in vitro studies, thiram did not significantly affect the growth and multiplication of* P. fluorescens *CP 8-3. Thus, the combination treatment of thiram @ 1.5 g kg^{-1} seed along with the bacterial antagonist (CP 8-3) can be used for effective control of the disease.*

Keywords: *Pseudomonas fluorescens, Sclerotium rolfsii*, collar rot, thiram, Annigeri, synergism

Introduction

Collar rot caused by *Sclerotium rolfsii* Sacc. is an important disease worldwide affecting chickpea production drastically. Control of soil borne diseases using host plant resistance has not been very effective. With the increasing concern of environmental pollution by application of pesticides, major efforts are being made to develop environment-friendly methods of plant disease control. Thus, considerable emphasis is placed to use fungal and bacterial antagonistic organisms, either alone or in combination with fungicides, for the control of soil/seed borne diseases (Muthamilan and Jeyrajan, 1996; Singh *et al.*, 2000). Hence, the present study was aimed at evaluating some bacterial antagonists against *S. rolfsii* in the greenhouse and field conditions.

Materials and methods

Two bacterial strains, *Pseudomonas fluorescens* Migula CP 8-2 and *P. fluorescens* CP 8-3, which were isolated from chickpea seed during seed health testing in 1999, were used in green house and field experiments @ 10^8 CFU ml^{-1} seed^{-1}.

Greenhouse evaluation (1999 & 2000)

Ten seeds of cv. Annigeri (collar rot susceptible) were sown in 15 cm diameter plastic pot containing soil infested with *S. rolfsii* in each treatment. There were six treatments (two bacterial strains alone, each of two bacterial strains in combination with thiram @1.5 g kg^{-1} seed, thiram alone @ 1.5g kg^{-1} seed and untreated control). Carboxy methyl cellulose (CMC 0.1%) was used as adhesive. All treatments were replicated four times. The pots were maintained in the greenhouse at a temperature ranging from 18°C (night) to 30°C (day). Observations on collar rot incidence were taken at 7, 14 and 21 days after sowing in both the years.

Field evaluation

A field experiment with susceptible cv. Annigeri (*Desi*) was conducted during 1999-2000 and 2000-2001 post rainy season in a collar rot sick-plot. There were nine treatments: two bacterial strains alone, each of the two bacterial strains in combination with thiram at 1.5 and 3 g kg^{-1} seed, thiram alone at 1.5 and 3 g kg^{-1} seed, and an untreated control. Each treatment was sown in two rows of 4 m each, and 40 seed were sown in each row. There were three replications arranged in Randomised block design (RBD). The observations on disease incidence were taken at 7, 14 and 21 days after sowing.

Survival and multiplication of *P. fluorescens* in the presence of thiram

Shake culture. One loopfull of actively growing culture of *P. fluorescens* CP 8-3 was added to each conical flask containing 50 ml nutrient broth with three concentrations of

thiram (@ 0.75, 1.5, and 3g per litre) and nutrient broth alone. Each treatment had three replications. All flasks were kept on a shaker at 30°C for 10 days. The bacterial population in all the treatments was counted at 2, 5 and 10 days intervals by dilution plate method using nutrient agar plates.

Nutrient agar plate culture

Nutrient agar plates containing three different concentrations of thiram (0.75, 1.5 and 3g l^{-1} medium) along with control were prepared. Actively growing culture of *P. fluorescens* was poured in five different wells of pre-sterilized pin inoculator (Josey *et al.*, 1979) that allowed transfer of uniform number of cells of a given culture to any number of plates desired. Each well was treated as a replication. The strain from the pin inoculator was inoculated on to three plates each of the above treatments. The plates were incubated at 30°C. Colony size (mm) was compared at eight different intervals to know the multiplication of bacterium.

Results and discussion

Greenhouse evaluation for the control of collar rot

Greenhouse studies revealed that the lowest level of collar rot incidence was recorded when thiram was applied in combination with *P. fluorescens* CP 8-3 (57.6 % collar rot against 95.6 % in control) in 1999, whereas 64.8 % in 2000. The two bacterial strains alone and *P. fluorescens* CP-8-2 in combination with thiram also gave significant reduction in collar rot (Table 1).

Table 1. Effect of *Pseudomonas fluorescens* strains and thiram on the incidence of chickpea collar rot in greenhouse during 1999 and 2000

	Per cent disease incidence (Mean)	
Treatment	1999	2000
P. fluorescens CP8-2	66.4	94.7
P. fluorescens CP8-3	73.0	88.9
Thiram 3.0 g	97.0	82.1
P. fluorescens CP8-2+CMC+Thiram 1.5g	76.4	64.7
P. fluorescens CP8-3+CMC+Thiram 1.5g	57.6	64.8
Control	95.6	88.9
SE ±	8.5	5.2

Field evaluation of bacterial strains against collar rot

The two bacterial strains in combination with thiram at both the rates of application gave significant reduction in collar rot incidence in both the cultivars (Table 3). The disease incidence in these treatments ranged from 7.3 to 15.5 % in Annigeri compared to 25.2 and 39.7 % in the two controls, respectively. The two bacterial strains alone and thiram alone at both the rates of applications were not effective.

Effect of thiram on multiplication of P. fluorescens CP 8-3

In shake culture

The bacterium reached maximum growth (8.1 log 10) within two days and remained high upto day five (8.1 log 10) in the absence of thiram. The bacterial population reduced (7.6 log 10) on day 10, but not significantly (Table 2). Addition of thiram reduced the growth of the bacterium. But the reduction was not significant at 0.75 g l^{-1} thiram. Significant reduction was noticed at day five and day 10 at 1.5-g l^{-1} thiram. Surprisingly, the count was high (5.33 log10) despite presence of 3.0 g l^{-1} thiram (Table 2). This strongly suggests the tolerance of the bacterium to thiram.

Table 2. Influence of Thiram on multiplication of *P. fluorescens* CP 8-3

	Numbers of colonies ml-1 broth (1og 10) Growth period (days)			
Treatment	2	5	10	Mean
Control	8.1	8.1	7.6	7.9
0.75 g l-1 Thiram	7.9	7.9	7.2	7.7
1.5 g l-1Thiram	8.1	5.9	5.3	6.4
3.0 g l-1 Thiram	7.2	5.3	5.3	6.0
LSD		0.5 (0.5)*		
Mean	7.8	6.8	6.4	
LSD		0.2		

* Parenthesis has LSD for comparing means of the same level of treatments.

In nutrient agar plate culture

Initial growth was visible within one day in control plates, while it took two days in the plates containing 0.75 g l^{-1} and 1.50 g l^{-1} of thiram, and six days in the plates with 3.0 g l^{-1} thiram. Maximum colony growth was achieved within three days in control, five days at 0.75 g l^{-1} and 1.50 g l^{-1} thiram and ten days at 3.0 g l^{-1} thiram. In the presence of thiram (even at 0.75 g l^{-1}) colony size was significantly less than control up to day 3.

The wide host range, saprophytic growth habit and unavailability of high level of genetic resistance have been the major limitations to the control of *S. rolfsii*. Although, *Trichoderma harzianum* has been reported to control this

Table 3. Effect of *P.fluorescens* with thiram on the incidence of collar rot of chickpea in sick-plot condition during 1999-2000 and 2000-2001

Treatments	Annigeri Disease (%) *
P. fluorescens CP8-2	19.8
P. fluorescens CP8-2+Thiram 1.5 g	9.6
P. fluorescens CP8-2+Thiram 3.0g	8.1
P. fluorescens CP8-3+Thiram 1.5g	15.5
P. fluorescens CP8-3+Thiram 3.0g	7.3
Thiram 1.5g	10.5
Thiram 3.0 g	19.5
P. fluorescens CP8-2	17.9
Control	25.2
SE	3.6

* Means of two experiments

pathogen in greenhouse and field tests (Chet, 1990), there has hardly been any success in using these results commercially (Campbell, 1994). This study has shown that the bacterial strains that were effective in inhibiting the fungal growth under *in vitro* conditions were not so effective in controlling the disease in pot conditions. However, the combined treatment with thiram and the bacterial strains was more effective than their individual applications. This clearly demonstrates the synergism between the bacterial strains and thiram. The existence of synergism was further confirmed by the normal multiplication and growth of the bacterium in the presence of thiram (Table 2). Combination of *T. harzianum* with captan has been used to control *Verticillium dahliae* in potato, which resulted in considerable increase in yield (Ordentlich *et al.*, 1990).

Despite synergism only an acceptable level of collar rot control was obtained in the field study conducted in a disease sick plot apparently having very high level of inoculum. Such levels of inoculum are unlikely to occur in farmers' fields. Therefore, the combined treatment is likely to give a high level of control under farmers' field conditions. Also, the combined use required half the quantity of thiram (1.5g kg^{-1}) to achieve maximum level of control.

The antagonistic activity of biocontrol agents depends upon their survival in the environment and on several other factors particularly its interaction with other soil microorganisms. Due to this reason the results from *in vitro* tests may not be correlated to field results, as observed in this study. Combined use of fungicide and microbial agent may be a more viable strategy to control a given disease provided the agent is tolerant to the level of prescribed fungicide.

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