MANAGEMENT STUDIES ON TOMATO DAMPING-OFF WITH NATIVE ANTAGONISTS

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Received-12.06.2015, Revised-20.06.2015

Abstract: Plant disease management with bio agents is a non chemical and environmental safe method in agriculture**.** Tomato damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. is one of the most dreadful diseases. Six isolates of *P. aphanidermatum* were collected from tomato nurseries of different geographical areas in Andhra Pradesh and designated as CTR_1, CTR_2, KDP_1, KDP_2, KNL_1 and KNL_2. Native *Trichoderma harzianum* and *Pseudomonas fluorescens* were isolated from tomato rhizosphere using selective media. These two native bioagents were identified upto species level based on morphological characters. Among the two native antagonists, *T. harzianum* recorded maximum per cent inhibition on all isolates of *P. aphanidermatum*. Maximum inhibition was observed in CTR_2 when *T. harzianum* was used while *P. fluorescens* recorded maximum inhibition on KDP_2 *in vitro*. *In vivo* studies revealed that seed treatment with combination of *T. harzianum* and *P. fluorescens* was found to be effective in controlling pre and post-emergence damping-off.

Keywords: *T. harzianum; P. fluorescens;* Tomato Damping – off; *P. aphanidermatum*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the most versatile vegetable crop grown in almost all the countries. There are over 20 diseases of tomato reported either in nursery, on the standing crop or during post harvest activities. Among these, damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. is one of the most dreadful diseases and is particularly severe in densely crowded nurseries. The disease was quite severe killing 10-15% of the tomato seedlings in nurseries (Govindappa and Grewal, 1965). The ubiquitous fungus, *P. aphanidermatum* was more common in both summer and winter grown tomato nurseries, causing losses to an extent of 50 per cent (Bisht *et al.*, 1997). Since this disease mostly affects nursery, proper management at nursery stage is very critical to ensure a healthy crop in the main field. Any negligence in the management of this disease at juncture, may jeopardise the cultivation of tomato crop totally as it literally wipes out the entire nursery. Crop losses inflicted by soil-borne pathogens continue to increase and become a limiting factor in stabilising or maximising crop yields on a world-wide basis.

The control measures available today including fungicides are not enough to the realistic elimination of soil-borne plant pathogens. The ill effects of escalated use of potentially hazardous pesticides like environmental pollution, increased cost of application and pathogen resistance lead to a drastic shift in the management strategies towards biological control of plant pathogens as an alternative to or as a part of IPM system for disease control (Baker and Snyder, 1965). These biocontrol methods can be successfully used in modern agriculture, especially with the native antagonists. Chemical seed treatment can protect the crop only at the early stage of its growth. Antagonists applied to seeds were found to colonize the rhizosphere and offer protection against soil borne pathogens (Muthamilan, 1989; Selvarajan, 1990; Turner and Backman, 1991).

Hence there is a need to screen *Trichoderma* and *Pseudomonas* populations of rhizosphere soil of tomato to identify effective ones against the test pathogen.

MATERIAL AND METHOD

Isolation of soil microflora: The soil microflora was isolated by serial dilution pour plate technique (Johnson and Curl, 1972) using selective media. *Trichoderma* spp. were isolated on *Trichoderma* selective medium (Elad and Chet, 1983) while *P. fluorescens* was isolated on King's B selective medium. The dilution used for isolation of *Trichoderma* spp was 10^{-4} and for *P. fluorescens* was 10^{-6}.

Trichoderma spp. were identified based on mycological keys described by Barnett and Hunter (1972), whereas *P. fluorescens* was identified based on Bergey's manual of determinative bacteriology (Holt *et al.*, 2000). *Trichoderma* spp. were maintained on PDA and *P. fluorescens* was maintained on nutrient agar medium by periodical transfer.

***In vitro* screening:** Dual culture technique was used to screen *Trichoderma* spp. and *P. fluorescens* against all the six isolates of *P. aphanidermatum*, *Trichoderma* and *P. fluorescens* were screened

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following the procedures of Khara and Hadwan, 1990; Vidhyasekaran *et al.,* (1997).

Three replications were maintained per treatment. The plates were incubated at 28 ± 2°C for 4 days and the zone of inhibition was measured. The per cent inhibition was calculated by using the following formula.

$$\% \text{ inhibition of } P. \text{ } aphanidermatum = \frac{\text{Growth of } P. \text{ } aphanidermatum \text{ in control plate} - \text{Growth of } P. \text{ } aphanidermatum \text{ in the presence of bio-agent}}{\text{Growth of } P. \text{ } aphanidermatum \text{ in control plate}} \times 100$$

Pot culture studies: The potential native antagonists were screened alone and in combination under glasshouse conditions against damping-off disease. 15 cm diameter pots were filled with 2 kg of sterilized soil. The virulent strain of *P. aphanidermatum* obtained was mass multiplied on the sorghum grains for 15 days. The multiplied inoculum was mixed with sterilized potting medium @ 100 g/ kg of soil. Then the pots were watered to activate the growth of the pathogen. After 2 days, tomato seeds treated with *Trichoderma* and *P. fluorescens* were sown @ 25 seeds per pot. Metalaxyl as seed treatment @ 6 g/kg of seed was included as a standard treatment for comparision. Pre-emergence and post-emergence damping-off disease incidence were recorded at 7 days and 25 days respectively after sowing.

The following treatments were imposed

T_1 - Seed treatment with *T. harzianum*-1 (T_1)
T_2 - Seed treatment with *P. fluorescens*
T_3 - Combination of ($T_1 + T_2$)
T_4 - Seed treatment with metalaxyl @ 6 g/kg
T_5 - Pathogen inoculated (control)
T_6 - Pathogen un-inoculated (control)

The *T. harzianum* were applied by the seed treatment method described by Syamasundar Reddy (1999). *P. fluorescens* was applied following the procedure given by Ramamoorthy *et al.* (2002). Seed treated with metalaxyl (ridomil MZ 72% WP) @ 6 g/kg of seeds included as a standard treatment for comparison.

Per cent disease incidence (PDI) was calculated using the following formula.

$$PDI = \frac{\text{Total no. of seeds sown - Healthy seedlings}}{\text{Total no. of seeds sown}} \times 100$$

RESULT AND DISCUSSION

Six isolates of *P. aphanidermatum* isolated from the infected tomato plants were identified and maintained on PDA medium. These isolates were proved to cause damping-off of tomato as it could be reisolated from infected seedlings in the pathogenicity test.

In the present study, *T. harzianum* and *P. fluorescens* were isolated from tomato rhizosphere using selective media. These antagonists were identified upto species level based on morphological characters (Subramanian, 1971).

T. harzianum and *P. fluorescens* were screened against all isolates of *P. aphanidermatum*. Among the two antagonists, *T. harzianum* recorded maximum per cent inhibition compared to *P. fluorescens* on all isolates of the pathogen. Among the six isolates of *P. aphanidermatum*, CTR_2 was more sensitive to *T. harzianum* and CTR_1 was less sensitive. In case of *P. fluorescens*, KDP_2 was more sensitive, while the isolate CTR_1 was found to be the least sensitive. The results indicate that *T. harzianum* is a destructive mycoparasite on *P. aphanidermatum* isolates (Table 1). This could be due to coiling of *Trichoderma* around the hyphae of *Pythium* isolate, CTR_2 while less penetration in other isolates. Similar observations were made by Vijaya Krishna Kumar (1997) who isolated different rhizosphere mycoflora from tomato plants and tested them against *P. aphanidermatum*. Among them *T. harzianum* was found to be more effective. *In vitro* screening of *T. harzianum* and *P. fluorescens* also reported by Swant and Mukhopadhyay (1990); Pratibha Sharma *et al.* (2003); Ongena *et al.,* 1999 and Ramamoorthy *et al.* (2002).

In vivo studies revealed that pre and post-emergence damping-off was less in the treatments having seed treatment with *T. harzianum* and *P. fluorescens* individually and in combination. Seed treatment with Ridomil MZ also controlled pre and post-emergence damping off. Among all the treatments, seed treatment with combination of *T. harzianum* and *P. fluorescens* was effective and recorded the least incidence in pre and post - emergence damping-off disease (Table 2).

The effectiveness of *T. harzianum* as seed treatment for the control of pre & post emergence damping-off caused by *Pythium* was reported by several workers (Krishna Moorthy and Bhaskaran (1990); Rama moorthy, *et al.,* (2002); Rahman *et al.,* 2003). The use of antagonistic microflora identified in this study offer a cheaper and environmentally safer alternative to the use of fungicides for seed treatment.

Table 1. Efficacy of antagonists against *P. aphanidermatum* isolates by dual culture technique

Soil microflora	Isolates of *P. aphanidermatum*											
	CTR_1		CTR_2		KDP_1		KDP_2		KNL_1		KNL_2	
	*Fungal growth (mm)	*% inhibition	Fungal growth (mm)	% inhibition	Fungal growth (mm)	% inhibition	Fungal growth (mm)	% inhibition	Fungal growth (mm)	% inhibition	Fungal growth (mm)	% inhibition
Trichoderma harzianum isolate-1	27.0	69.2 (56.3)	10.3	88.5 (70.2)	18.1	79.8 (63.3)	12.6	86.0	15.5	82.7 (65.4)	24.2	73.1 (58.7)
P. fluorescens	28.8	67.9 (55.1)	13.5	85.0 (67.2)	19.8	77.9 (61.9)	11.6	87.1 (68.9)	16.8	81.3 (64.4)	25.2	72.0 (58.0)
Control	90.0	0.00 (0.00)	90.0	0.00 (0.00)	90.0	0.00 (0.00)	90.0	0.00 (0.00)	90.0	0.00 (0.00)	90.0	0.00 (0.00)
SEm (±)		1.92		0.48		0.77		0.61		0.79		0.57
CD (5%)		6.63		1.66		2.66		2.13		2.74		1.96

* Mean of three replications
Figures in parentheses are angular transformed values

Table 2. Effect of different treatments on pre and post - emergence damping - off at 7 and 25 days after sowing (DAS)

Treatments		*Pre-emergence (7 DAS) damping-off		*Post-emergence (25 DAS) damping-off	
		(Per cent disease incidence)	Per cent inhibition over control	(Per cent disease incidence)	Per cent inhibition over control
T_1	Seed treatment with *T. harzianum*	9.2 (17.6)	62.2 (52.0)	30.5 (33.5)	54.3 (47.4)
T_2	Seed treatment with *P. fluorescens*	10.0 (18.4)	59.1 (50.24)	36.2 (36.9)	45.8 (42.5)
T_3	Seed treatment with combination of *T. harzianum* and *P. fluorescens*	5.7 (13.8)	76.5 (61.0)	19.3 (26.0)	71.1 (57.4)
T_4	Seed treatment with Metalaxyl (6 gm/kg)	9.0 (17.4)	63.2 (52.6)	26.0 (30.6)	61.0 (51.3)
T_5	Pathogen inoculated control	24.5 (29.6)	0.0 (0.0)	66.8 (54.8)	0.00 (0.0)
T_6	Pathogen un-inoculated control	0.0 (0.0)	100.0 (90.0)	0.00 (0.00	100.0 (90.0)
	SEm (±)	0.16	-	0.20	-
	CD at 5%	0.48	-	0.62	-

* Mean of three replications
Figures in parentheses are angular transformed values

ACKNOWLEDGEMENT

The authors are grateful to Acharya N.G. Ranga Agricultural University, Hyderabad for the financial assistance.

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