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Biological control of onion basal rot (*Fusarium oxysporum* f. sp. *cepae*) by combined application of fungal and bacterial antagonists

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ABSTRACT : Fungal antagonists *Trichoderma viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* and the bacterial antagonists, *Pseudomonas fluorescens* and *Bacillus subtilis* were effective against *Fusarium* f. sp. *cepae* in *in vitro* conditions. Combined seed treatment of *T. viride* + *P. fluorescens* reduced the onion basal rot incidence both in pot and in field conditions.

KEY WORDS : Antagonist, *Bacillus subtilis*, basal rot, *Fusarium oxysporum* f. sp. *cepae, Pseudomonas fluorescens, Trichoderma* spp.

Basal rot, a serious disease of onion, caused by *Fusarium oxysporum* f. sp. *cepae* (Hanz) Snyd. and Hans. occurs both in the nursery and in the field. In some situations the fungus is responsible for total failure of onion seedlings in nursery. Biological control of plant diseases is gaining importance as an alternative means of crop managment. Damping-off of onion caused by *F. oxysporum* f. sp. *cepae* was significantly suppressed by *Pseudomonas cepacia* (Kawamoto and Lorbeer, 1976). *Gliocladium roseum, T. viride* and *T. harzianum* were reported to be antagonistic to onion basal rot pathogen, *F. oxysoporum* f. sp. *cepae* in *in vitro* and *in vivo* conditions (Rod, 1984). It was also recorded that *P. fluorescens* and *P. putida* minimised the disease incidence in tomato caused by *Fusarium* spp. and *Pythium* spp. by reducing the mycelial growth and inhibiting the spore germination (Formmell and Pazos, 1993).

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In the present investigation, efficiency of *T. viride* and *P. fluorescens* was tested against onion basal rot pathogen *F. oxysporum* f. sp. *cepae* under laboratory, glass house and field conditons.

MATERIALS AND METHODS

Fusarium f. sp. *cepae* was isolated from bulbs showing typical basal rot symptoms in Potato Dextrose Agar (PDA) medium. Pure culture of the pathogen was obtained by single hyphal tip method (Rangaswami, 1972). The culture was multiplied in sand-maize medium and utilised for the evaluation of different species of antagonists (Muthuswamy, 1972).

The antagonists available in the Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore were used in the present study.

In vitro evaluation of antagonists

The antagonistic effect of *T. viride, T. harzianum, T. koningii, T. pseudokoningii, T. hamatum* and four strains of *P. fluorescens* e.g., pf-1, pf-2, pf-3, pf-4 and *Bacillus subtilis* were tested against *F. oxysporum* f.sp. *cepae* by dual culture technique (Dennis and Webster, 1971). The growth of *F. oxysporum* f. sp. *cepae* was measured 5 days after incubation at room temperature.

Evaluation of antagonists in pot culture

The pathogen multiplied in sand-maize medium was incorporated into the soil in pots (60 x 45 cm) at 5 per cent (w/w). The seeds treated with talc based formulation of fungal and bacterial antagonists at 4g/kg were sown in pots preinoculated with *F. oxysporum* f. sp. *cepae* 10 days before sowing. Control was maintained without antagonists treatment. Four replications were maintained in each treatment. Per cent seed germination was recorded 10 days after sowing and the basal rot incidence was recorded at 15, 30, 45 and 60 days after sowing (DAS).

Field evaluation of antagonists

A field experiment was carried at the Horticultural College and Research Institute, TNAU, Coimbatore during May-June, in a basal rot sick plot. The experiment was conducted in a randomised block design (RBD) with 3 replicates in 1 x 1m plot. Just before sowing, seed treatments with talc based formulation of fungal and bacterial antagonists were given at 4g/kg. Carbendazim at 4g/kg was used for comparison. The germination percentage of treated seeds was recorded 10 days after sowing. Basal rot incidence was recorded 15, 30, 45 and 60 days after sowing. The data collected were subjected to analysis of variance.

RESULTS AND DISCUSSION

In vitro evaluation of antagonists

The fungal antagonists *viz.*, *T. viride, T. harzianum* and *T. pseudokoningii* inhibited the growth of onion basal rot pathogen very effectively under *in vitro*

conditions. *Trichoderma viride* reduced growth of the pathogen to a maximum extent (53%) as compared to *T. harzianum* (48%) and *T. pseudokoningii* (47%). *Trichoderma hamatum* was the least effective (31%) in inhibiting the growth of *F. oxysporum* f. sp. *cepae*. Chung and Choi (1990) reported effective inhibition of *R. solani* and *F. oxysporum* f. sp. *sesami* under *in vitro* conditions by *T. viride*. Muthuraman and Sekar (1993) found that *T. viride* and *T. harzianum* inhibited the growth of *F. oxysporum* f. sp. *cepae*. The bacterial antagonists *P. fluorescens* strain-1 caused maximum reduction (55%) in growth of onion basal rot pathogen compared to other strains of *P. fluorescens* and *B. subtilis* (Table 1). Muthamilan (1994) reported that *P. fluorescens* isolates were effective against *F. oxysporum* f. sp. *ciceri* both under *in vitro* and *in vivo* conditions.

Evaluation of antagonists in pot culture

In pot culture studies, combination of *T. viride* and *P. fluorescens* recorded highest seed germination (88%) followed by *T. harzianum* + *P. fluorescens* (81%) compared to control (38%) (Table 2).

Table 1. Efficacy of fungal and bacterial antagonists against *F. oxysporum* f. sp. *cepae* under *in vitro* conditions

Treatment	Growth of *F. oxysporum* (mm)*	Growth reduction over control (%)
Fungal Antagonists		
Trichoderma viride	23.75	53.88
T. harzianum	26.50	48.54
T. pseudokoningii	27.25	47.09
T. koningii	28.50	44.66
T. hamatum	35.25	31.55
C D (P = 0.05)	1.35	
Bacterial antagonists		
P. fluorescens strain 1	23.00	55.33
P. fluorescens strain 2	27.25	47.09
P. fluorescens strain 3	29.50	42.72
P. fluorescens strain 11	32.25	37.38
B. subtilis	31.50	38.83
Control	51.50	--
C D (P=0.05)	3.82	

* After 5 days of inoculation

Trichoderma viride + *P. fluorescens* registered minimum disease incidence of 16, 24, 29 and 31 per cent as against 75, 84, 92 and 94 per cent in control 15, 30, 45 and 60 days after sowing, respectively (Table 2).

viride (76%). The per cent seed germination in *T. viride* was on par with carbendazim. Control recorded the least germination (36%) (Table 3). At all stages minimum disease incidence was noted in *T. viride* + *P. fluorescens* treated plots,

Table 2. Effect of antagonist on seed germination and onion basal rot incidence under pot culture conditions

Treatment	Per cent germination	Per cent disease incidence (DAS)			
		15	30	40	60
T. viride	78.00	28.45	36.00	42.45	45.68
	(62.14)	(32.16)	(36.84)	(40.66)	(42.52)
T. harzianum	73.00	33.45	43.15	48.53	50.68
	(58.77)	(35.56)	(40.91)	(44.14)	(45.27)
P. fluorescens	67.00	36.98	46.70	52.08	54.23
	(54.97)	(37.38)	(43.10)	(46.19)	(47.26)
B. subtilis	55.00	55.80	65.53	70.00	72.05
	(47.88)	(48.34)	(54.05)	(56.75)	(58.10)
T. viride +	88.00	16.30	24.90	29.20	31.35
P. fluorescens	(69.86)	(23.73)	(30.00)	(32.68)	(34.04)
T. harzianum +	81.00	24.38	31.90	36.20	38.35
P. fluorescens	(64.24)	(29.55)	(34.38)	(37.00)	(38.26)
B. subtilis +	66.00	37.60	50.60	57.05	61.35
T. viride	(54.34)	(37.81)	(45.34)	(49.06)	(51.56)
B. subtilis +	58.00	44.60	55.40	61.55	65.85
T. harzianum	(49.61)	(41.80)	(48.10)	(51.68)	(54.24)
Control	38.00	75.00	84.67	92.20	94.35
	(38.05)	(60.02)	(67.00)	(73.90)	(76.25)
C D (P = 0.05)	4.54	4.06	2.87	1.76	0.67

Figures in the parenthesis are arcsine values
DAS = Days after sowing

Field evaluation of antagonists

In the field trial onion seeds treated with *T. viride* + *P. fluorescens* recorded highest germination (86%) followed by *T. harzianum* + *P. fluorescens* (80%) and *T.*

compared to all other treatments. In control the disease incidence was 72, 82, 89 and 94 per cent 15, 30, 45 and 60 DAS, respectively.

Seed treatment with *P. cepacia*

Table 3. Effect of biocontrol agents on germination and onion basal rot incidence under field conditions

Treatment	Per cent germination	Per cent disease incidence (DAS)			
		15	30	40	60
T. viride	76.70 (61.17)	28.41 (32.20)	34.45 (35.95)	38.72 (38.49)	40.89 (39.75)
T. harzianum	71.06 (57.48)	34.78 (36.13)	41.87 (40.32)	46.33 (42.91)	49.09 (44.47)
P. fluorescens	66.16 (54.76)	36.57 (39.00)	47.18 (43.47)	51.91 (46.11)	54.93 (47.83)
B. subtilis	53.48 (47.01)	54.59 (47.62)	63.25 (52.69)	69.42 (56.44)	72.90 (58.59)
T. viride + *P. fluorescens*	86.34 (68.32)	19.18 (25.91)	23.19 (28.52)	25.35 (28.50)	26.17 (30.84)
T. harzianum + *P. fluorescens*	80.58 (63.85)	23.62 (29.06)	28.67 (32.37)	31.37 (34.06)	33.40 (35.52)
B. subtilis + *T. viride*	60.76 (51.20)	46.19 (42.84)	54.26 (47.43)	59.58 (50.55)	62.60 (52.49)
B. subtilis + *T. harzianum*	57.42 (49.27)	50.26 (45.15)	58.59 (50.00)	64.30 (53.33)	67.90 (55.49)
Control	36.91 (36.08)	72.06 (58.20)	81.21 (65.05)	89.23 (70.85)	94.29 (76.19)
C D (P = 0.05)	1.30	1.7	1.64	3.07	1.56

Figures in the parenthesis are arcsine values

DAS = Days after sowing

reduced the damping-off disease in onion caused by *F. oxysporum* f. sp. *cepae* (Kawamoto and Lorbeer, 1976). Papavizas and Lewis (1983) reported that the incidence of *F. oxysporum* f. sp. *pisi* in pea was reduced by seed treatment with either *T. harzianum* or *T. viride* and reduction in disease incidence was on par with captan and metalaxyl compound.

The results indicate that combined application of *T. viride* and *P. fluorescens* to onion seeds could give protection to onion bulbs from basal rot pathogen.

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