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Screening and selection of potential *Trichoderma* isolates for the control of cotton seed rot and damping-off

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ABSTRACT: **Out of twelve *Trichoderma* isolates tested, cotton isolate *T.viride* (97) exhibited the fastest growth rate and strong antagonism against *Rhizoctonia solani in vitro* recording 56.2 per cent inhibition in dual culture. All the isolates tested enhanced cotton seed germination when treated with mycelial suspensions of bioagents *in vivo*. The disease incidence in bioagent treatments ranged between 14.8 to 32.4 per cent, while pathogen treatment recorded 82 per cent. Three isolates have recorded seedling vigour index above 2000, which was much better than fungicide treatment (1471) in blotter tests (Roll Towel Method). *T.viride* (32) recorded the maximum seed germination (86.4 per cent) and no incidence of post -emergence mortality under green house conditions. The seedling vigour index in bioagent treatments ranged between 1004 and 1866.**

KEY WORDS: Biocontrol, Cotton, Damping-off, *Rhizoctonia solani, Trichoderma*

INTRODUCTION

Damping-off caused by *Rhizoctonia solani* Kuhn is a serious threat to cotton seedlings worldwide. Though the exact losses caused by this disease have not been assessed systematically in India, Vasudeva (1942) has reported a loss of about three per cent under normal conditions. In extreme cases the disease bodies may be as great as ninety per cent of the crop. During the last decade, *Trichoderma* species have shown tremendous potential of controlling several plant diseases especially the soil borne diseases. Papavizas (1985) has published an exhaustive review on the ecology, biology and biocontrol potential of *Trichoderma* and *Gliocladium*. The use of antagonistic fungi *Trichoderma* has been extensive against several soil borne pathogens of major crops of India (Mukherjee and Mukhopadhyay, 1996; Mukherjee, 1997;). There are several reports on the use of *Trichoderma* for biological control of R. *solani* in cotton (Sreenivasaprasad and Manibhushanrao, 1990; Onan *et al.*, 1998; Jakhar *et al.*, 1998), French bean (Hazarika and Das, 1998), damping-off of cardamom (Bhai *et al.*, 1999), damping-off of chilli (Harris, 1999), stem-rot in soybean (Dutta and Das, 1999) and seed-rot and damping-off of chickpea (Prasad and Rangeshwaran, 2000). In the present investigation, we report biocontrol potential of *Trichoderma* species for the management of cotton damping-off caused by *R. solani*.

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MATERIALS AND METHODS

Isolation of Pathogn and Bioagents

Rhizoctonia solani Kuhn the incitant of cotton seedling disease (seed rot and damping-off) was isolated from infected cotton roots, purified by single hyphal tip method and was maintained on Potato Dextrose Agar slants. Five isolates of *Trichoderma* were isolated from the rhizosphere of healthy cotton from different regions. Seven isolates, viz., *Trichoderma harzianum* (PDBCTH 10), *T. harzianum* (PDBCTH 15), *T. viride* (PDBCTV 6), *T. viride* (PDBCTV 23), *T. viride* (PDBCTV24) *T. viride* (PDBCTV 32) and *T. virens* (PDBCTVS 12) were obtained from Project Directorate of Biological Control culture collection. All the isolates were maintained on PDA slants.

In vivo method of screening of *Trichoderma* spp.

Seeds of cotton cv. RCH 2 were surface disinfected with 2 per cent sodium hypochlorite solution followed by serial washings with sterile water. Surface disinfected seeds were first inoculated with mycelial suspension by scrapping three days old mycelium of *Rhizoctonia* with sterile scalpel and dissolving in sterile distilled water (2 x 10^3 cfu/ml.) followed by treatments with conidial suspensions of different *Trichoderma* isolates containing 2 x 10^3 cfu/ml. One set of seeds was treated with fungicide (Captan 4gm/kg of seed). Surface disinfected seeds inoculated with pathogen alone served as control.

Three replications of 50 seeds each were maintained. The treated seeds were placed on moist blotter sheets following ISTA (1976) with certain modifications. After placing seeds on blotter sheets, another moist blotter sheet was placed on seeds to cover them and then rolled and three such rolled blotter bundles were tied as a single bundle and kept in growth chambers at 25°C and 80 per cent RH. Moisture in blotter bundles was maintained by applying sterile water at regular intervals.

The data on per cent germination, root and shoot length was recorded on 10^{th} day and vigory index was calculated following Abdul-Baki and Anderson (1973) by multiplying germination percentage to the sum of root and shoot length.

Greenhouse pot tests

Soil mixed with Farmyard manure (FYM) was sterilized in autoclave and filled in three Kg capacity mud pots. *Rhizoctonia solani* was multiplied in Petri-plates containing PDA. Ten-day old cultures were scraped with a sterile scalpel and blended in a maxi with 250 ml of sterile water. After treating the seeds with pathogen suspension (2 x 10^3 cfu/ml.), seeds were treated with suspension of different isolates of *Trichoderma* (2x 10^3 cfu/ml.) and sown in pots. Seeds sown without *Trichoderma* served as control and one set of seeds treated with fungicide was also sown. Three replications of 25 seeds each were maintained for each treatment. Data on seed germination, seedling mortality, root and shoot length of seedlings were recorded on tenth day after sowing.

RESULTS AND DISCUSSION

The germination of seeds in all the treatments (79.9 to 90.2%) was significantly higher as compared to pathogen check (27.2%) (Table1). Maximum seed germination was observed with *T. viride* cotton isolate (32) while *T. virens* (PDBCTVS 12) recorded the minimum seed germination of 79.3 per cent.

There was a significant increase in shoot and root length in all bioagent treatments compared to pathogen check. *T. viride* isolate 12 recorded the maximum shoot length of 9.0 cm and root length of 16.4 cm followed by *T. hamatum* isolate 138. *T. viride* (PDBCTV 6) recorded the minimum shoot length of 5.6 cm and a root length of 11.6 cm. The seeds treated with pathogen alone registered 5.8 cm shoot length and 3.1 cm root length.

Seedling vigour index ranging from 1477.5 to 2100.6 was obtained with various bioagent treatments. Maximum vigour index (2100.6) was recorded in *T.viride* isolate 12 followed by *T. harzianum* (PDBCTH 10) recording 2062.6. Minimum vigour index of 1477.5 was recorded in *T.viride* isolate (PDBCTV 6). The pathogen check

Table 1. Effect of seed treatment with *Trichoderma* isolates on germination, seedling vigour index and disease incidence in cotton

Antagonist	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index	Disease incidence (%)
Trichoderma viride (PDBCTV 6)	85.9 (67.9)	5.6	11.6	1477.5	32.4(34.70)
T. viride (PDBCTV 23)	84.4 (66.7)	8.2	15.0	1958.1	17.9(25.0)
T. viride (PDBCTV 24)	80.6 (63.9)	7.8	13.5	1716.8	16.4(23.9)
T. viride (PDBCTV 32)	82.1 (65.0)	6.4	12.8	1576.3	17.5(24.7)
T. virens (PDBCTVS 12)	79.9 (62.9)	7.1	14.2	1689.1	21.3(27.5)
T. harzianum (PDBCTH 10)	86.3 (68.3)	8.6	15.3	2062.6	15.0(22.8)
T. harzianum (PDBCTH 15)	81.4 (64.5)	8.2	15.4	1921.0	22.2(28.1)
Cotton isolates	-	-	-	-	-
T. viride (12)	82.7 (65.4)	9.0	16.4	2100.6	14.8(22.6)
T. viride (32)	90.2 (71.8)	5.6	13.1	1686.7	18.5(25.5)
T. viride (97)	82.7 (65.4)	6.9	14.5	1769.8	19.1(25.9)
T. viride (115)	80.4 (63.7)	7.5	12.2	1583.9	18.4(25.4)
T. hamatum (138)	84.6 (66.9)	8.7	15.6	2055.8	16.0(23.6)
Fungicide-captan	86.5 (68.4)	7.3	9.7	1470.0	17.0(24.4)
Pathogen check	27. 2 (31.4)	3.8	3.1	187.7	82.0(64.9)
CD (P=0.05)	3.02	0.6	0.77	173.0	2.30

Figures in parentheses are angular transformed values.

recorded a vigour index of 187.7 only. All the bioagent treatments promoted significantly greater vigour index than fungicide treatment although the fungicide treatment gave better control of the disease. This indicates the possibility of *Trichoderma* inducing growth of cotton seedlings by one or more mechanisms. The same trend of enhanced growth in sunflower was reported by Siddique *et al.* (1998).

All bioagents and fungicide treatments significantly inhibited disease incidence when compared to pathogen check. The disease incidence ranged between 14.8 to 32.4 per cent in bioagent treatments. Lowest disease incidence of 14.8 and 15 per cent was recorded in *T. viride* isolate 12 and *T. harzianum* (PDBCTH 10) treatments, respectively. Fungicide and pathogen treatments recorded a disease incidence of 17 and 82 per cent, respectively.

The results represented in Table 2 show the bioefficacy of twelve *Trichoderma* isolates on the growth of cotton seedlings and damping-off incidence. All the bioagent treatments significantly reduced both pre- emergence and post- emergence mortality as compared to pathogen treatment. The pre-emergence mortality in bioagent treatments ranged from 17.5 to 32.3 per cent while it was 94 per cent in pathogen check. *T. harzianum* (PDBCTH 10) recorded the lowest pre- emergence mortality (17.5 per cent) followed by *T. harzianum* (PDBCTH 15) recording 18.3 per cent. Fungicide treatment recorded 28.2 per cent mortality. With regard to

post-emergence mortality, *T. viride* 12 recorded no incidence. The post- emergence mortality in bioagent treatments ranged from 0 to 14.8 per cent.

T. viride isolate 12 recorded the highest seed germination (86.4%) followed by *T. viride* (PDBCTV 32) 83.1 per cent. Minimum seed germination was obtained in *T. viride* (PDBCTV 24) treatment (67.4%) while it was 31.7 per cent in pathogen treatment. Regarding shoot length *T. viride 12* recorded the maximum shoot length of 8.4cm followed by *T. viride* (PDBCTV 23) recording 8.1cm (Table-2). All the bioagent treatments except *T. viride* (PDBCTV 24 and 32) gave better shoot length than fungicide treatment, which was 6.5cm. In root length also all the bioagent treatments gave enhanced root length ranging from 9.6 to 13.2 cm, which was significantly higher than fungicide treatment (8.7cm). All the bioagent treatments gave better seedling vigour index ranging from 1004.3 to 1866.2 while pathogen treatment recorded significantly less vigour index of 158.5. All the bioagents except *T. viride* (PDBCTV 24) yielded higher vigour index than the fungicide treatment which was 1234.2. The

Table 2. Effect of *Trichoderma* isolates on *R.solani* infection and growth of cotton seedlings in pot

Bioagents	Germination (%)	Pre-emergence mortality (%)	Post-emergence mortality (%)	Shoot length (cm)	Root length (cm)	Vigour index	Dry wt. of shoots (g)	Dry wt. of roots (g)
Trichoderma viride (PDBCTV 6)	75.2(60.1)	32.3(34.6)	9.1(17.6)	7.7	9.8	1316.0	0.52	0.38
T. viride (PDBCTV 23)	72.8(58.6)	24.6(29.7)	8.5(17.0)	8.1	11.9	1456.0	0.56	0.42
T. viride (PDBCTV 24)	67.4(55.2)	30.7(33.7)	14.6(22.5)	5.9	9.0	1004.3	0.47	0.36
T. viride (PDBCTV 32)	83.1(65.7)	21.4(27.6)	4.2(11.8)	6.4	11.4	1479.2	0.49	0.43
T. virens (PDBCTVS 12)	77.9(62.0)	19.8(26.4)	7.6(16.0)	6.8	10.1	1313.5	0.56	0.29
T. harzianum (PDBCTH 10)	80.5(63.8)	17.5(24.7)	11.1(19.5)	7.2	12.7	1602.0	0.63	0.57
T. harzianum (PDBCTH 15)	78.5(62.4)	18.3(25.3)	14.8(22.6)	7.4	11.3	1468.0	0.57	0.51
Cotton isolates	-	-	-	-	-	-	-	-
T. viride (12)	86.4(68.4)	19.1(25.9)	-	8.4	13.2	1866.2	0.71	0.68
T. viride (32)	74.3(59.5)	18.7(25.6)	10.8(19.2)	8.0	12.5	1523.2	0.62	0.55
T. viride (97)	78.7(62.5)	24.7(29.8)	9.2(17.7)	6.5	9.6	1267.1	0.48	0.39
T. viride (115)	80.2(63.6)	22.3(28.2)	4.3(12.0)	7.1	10.7	1427.6	0.52	0.32
T. hamatum (138)	69.6(56.5)	28.6(32.3)	8.7(17.2)	7.4	11.8	1336.3	0.52	0.47
Fungicide-captan	81.2(64.3)	28.2(32.1)	12.7(20.9)	6.5	8.7	1234.2	0.50	0.35
Pathogen check	31.7(34.3)	94.0(75.8)	6.0(14.2)	2.6	2.4	158.5	0.09	0.10
CD (P=0.05)	2.98	0.96	0.71	0.57	0.63	167.0	0.14	0.11

Figures in parentheses are angular transformed values.

phenomenon of bioagents recording higher vigour index than fungicide treatment implies growth promoting mechanism of bioagents. In blotter tests also the same trend was noticed. Based on the results, cotton isolates namely *T. viride* 12 and 32 and *T. harzianum* (PDBCTH 10) were selected as potential antagonists against *R. solani* in cotton.

The results of the present investigation suggested high degree of biocontrol potential of *Trichoderma* species against cotton damping-off caused by *R.solani*. However, further studies are required to develop suitable technology for the economic control of this dreaded disease under field conditions in different agro climatic regions.

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