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ORIGINAL ARTICLE

Biocontrol of damping off of chilli (*Capsicum annuum* L.) by *Trichoderma* species

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ABSTRACT

A series of laboratory and pot culture experiments in green house were conducted to evaluate the efficacy of native Trichoderma sp. isolates against Pythium aphanidermatum, the causative agent of pre-emergence seed rot and post emergence damping off of chilli. In all 13 isolates of Trichoderma were isolated of which six were of T. viride and seven were of T. harzianum. In dual culture testing of Trichoderma sp. along with Pythium aphanidermatum, it was observed that all the isolates of Trichoderma sp. inhibited the radial growth of pathogen. Three isolates of Trichoderma viride and two isolates of Trichoderma harzianum exhibited an inhibition zone of more than 12 mm and showed overgrowth, coiling, lysis and profuse sporulation. Non volatile antibiotics produced by Trichoderma sp. inhibited the growth of P. aphanidermatum. Trichoderma sp. were found to be effective in reducing the incidence of pre-emergence seed rot and post emergence damping off in pot culture experiments when applied as soil application as well as seed treatment, but later was more effective in controlling the disease and was at par with chemical seed treatment. While among isolates T. viride A 14 was found to be effective in controlling the diseases and was at par with fungicidal seed treatment

Key words: *Pre-emergence seed rot, Post emergence damping off, Pythium aphanidermatum, Trichoderma sp., Biological control*

INTRODUCTION

Damping off of chilli (*Capsicum annuum* L.) seedlings is one of the most common fungal disease in the Chilli growing tract of Dharmabad- Billoli region of Marathwada, Maharashtra. This disease accounts for severe mortality in the seedlings in nurseries, green houses, shade houses and on field. The pathogen attacks at the pre-emergence stage causing either seed rot or damping off of seedlings.

The disease is caused by *Pythium aphanidermatum* and it is well recorded from chilli growing areas of India and other parts of the world. The control of *Pythium* is difficult due its prolonged survival and its soil borne nature. Therefore the management of damping off disease not only in chillies but also in other crops poses a severe threat. The management of the disease by fungicide is difficult and expensive. Biological control offers a simple, cost effective and environmental friendly method as compared to fungicidal control. The establishment and persistence of the biocontrol agent after its introduction provides protection of the crop for a longer time. Moreover the prolonged use of fungicide for years together has lead to acquired resistance to these fungicides by the pathogen.

Trichoderma sp. are well known for their antagonistic nature towards a wide range of plant pathogenic fungi. Liamngee Kator et.al (2015) found that *Trichoderma harzianum* and *T. viride* possess potential antifungal activity and can be used for field application. They have proved to be reliable method of biological control for most of the economically important crops (Papavizas, 1985; Howell, 2002). Hence, a series of experiments were conducted in laboratory and green house to study effective fungal antagonist to control *Pythium aphanidermatum* causal organism of damping off of chilli.

MATERIAL AND METHODS

ISOLATION OF PATHOGEN:

Regular visits were made to nurseries, shade house and growers field around Dharmabad– Billoli region of Marathwada, Maharashtra, to collect diseased plant material. The pathogen was isolated

from diseased plant seedlings. Semi-permanent slides of fungi were prepared, isolated at appropriate stage of growth of the fungus and measurements of vegetative and reproductive structures etc. were taken. These were then compared with those recorded earlier and identification was confirmed. The cultures were preserved in PDA slants and were incubated at 27 ± 2 °C and sub culturing was done every 15 days.

ISOLATION OF BIOCONTROL AGENT:

During the regular visits healthy seedlings were also collected and rhizospheric soil was also taken. The root sections as well as the rhizospheric soil were plated for the isolation of fungi and bacteria present on rhizoplane and in rhizosphere respectively. Semi-permanent slides of fungi were prepared, isolated at appropriate stage of growth of the fungus and measurement of hyphae, condiophore, conidia etc. were taken. These were then compared with those recorded earlier and identification was confirmed (Samuels *et al*., 2004). The cultures were preserved on PDA slants and were incubated at 27±2 °C. Sub culturing was done every 15 days. In all 13 isolates of *Trichoderma sp.* were isolated from the rhizospheric soil and rhizoplane of healthy chilli seedlings. Out of 13 isolates six were of *T. viride* and seven were of *T. harzianum*.

ASSAY OF THE ANTAGONISTIC ACTIVITY:

The antagonist activity of the *Trichoderma sp.* against *Pythium aphanidermatum* was determined by dual culture technique (Dennis and Webster, 1971b) on PDA and the radial growth of the pathogen and the test fungi were measured at various intervals during 120 h of incubation period. The production of non volatile diffusible antibiotic by *Trichoderma sp.* was tested following standard procedure of Dennis and Webster (1971a).

RESULT AND DISCUSSION

Thirteen strains of *Trichoderma sp.* were isolated from various locations and were tested for their antagonism against *P. aphanidermatum*. They were tested to evaluate their effect on the radial growth of *P. aphanidermatum* in dual culture. It is evident from the results (Table 1) that the radial growth of *P. aphanidermatum* was adversely affected by the antagonist, isolates of *T. viride* and *T. harzianum*. Six isolates of *T. viride* and seven isolates of *T. harzianum* have significantly reduced the radial growth of pathogen. Three isolates of *T. viride* and two isolates of *T. harzianum* exhibited an antagonized zone of more than 12 mm width and showed overgrowth, coiling, lysis and profuse sporulation. The highest zone of inhibition of pathogen was caused by *T. viride* which was 15.5 mm and for *T. harzianum* 13.5 mm was the highest zone of inhibition.

Table 1: Effect of *Trichoderma* sp. isolates on the radial growth of *P. aphanidermatum* in dual culture

Sr. No	Isolate	Mean Radial Growth (mm/120 h)		Zone of inhibition
		Isolate	Pathogen	
1	*T.viride* A5	46.0	41.0	10.0
2	*T.viride* A10	43.3	57.0	12.0
3	*T.viride* A3	32.2	54.6	09.3
4	*T.viride* A11	30.5	57.1	14.2
5	*T.viride* A14	37.7	45.3	15.5
6	*T.viride* A7	38.2	51.7	9.2
7	*T. harzianum* D20	29.0	60.5	06.5
8	*T. harzianum* D17	35.5	49.0	12.7
9	*T. harzianum* D6	32.8	40.8	13.5
10	*T. harzianum* D2	31.2	55.4	07.3
11	*T. harzianum* D12	34.4	53.0	08.0
12	*T. harzianum* D15	32.5	54.7	10.5
13	*T. harzianum* D19	30.7	56.2	07.3
Control		-	70	-
SE		0.35	0.74	0.03
CD(P=0.05)		1.1	2.3	0.8

Antagonistic activity of *Trichoderma sp*. against *P. aphanidermatum* has been reported earlier by several workers (Sivan *et al*., 1984; Lifshitz *et al*., 1986; Gnanvel and Jayaraj, 2003). The mechanism of antagonism expressed by *T. viride*, in this study, against *P. aphanidermatum* is over growth, coiling and lysis (mycoparasitism). It is observed that *T. viride* grew the hyphae of *P. aphanidermatum* in dual culture, and inhibited further growth of pathogen on contact. It shows antagonism by over growth on the hyphae of *P. aphanidermatum* in dual culture. The growth and coiling of *T. viride* around the hyphae of *P. aphanidermatum* could involve antibiotic and enzyme production, which led to direct parasitism as well as lysis (D'Ercole *et al*., 1984).

The non volatile antibiotics produced by *Trichoderma sp.* inhibit the growth *P. aphanidermatum*. Inhibition of *P. aphanidermatum* was noticed in culture filtrate of all isolates of *T. viride* and *T. harzianum* (Table 2). The present study reveals that the toxic substances produced by isolates were also effective for inhibiting the pathogen and physical contact between isolates and pathogen may not be necessary for effective antibiosis (Narsimha Rao and Kulkarni, 2003). Several workers reported the inhibitory effects of both volatile and non-volatile substance produced by *Trichoderma* species on several soil borne pathogens (Dennis and Webster, 1971b; Upadhyay and Mukhopadhyay, 1983; Neelmegam, 2004). The control of *P. aphanidermatum* by *T. viride* and *T. harzianum* in this study was carried out through the antagonistic activities such as overgrowth, coiling, lysis and production of non-volatile antibiotics.

Table 2: Effect of non volatile substance of *Trichoderma sp* isolates on mean radial growth of *P. aphanidermatum*

Sr. No	Isolate	Mean Radial Growth of Pathogen (mm/120 h)
1	*T.viride* A5	48.44 (-30.85)
2	*T.viride* A10	44.35 (-36.71)
3	*T.viride* A3	54.36 (-22.42)
4	*T.viride* A11	51.40 (-26.57)
5	*T.viride* A14	39.48 (-43.60)
6	*T.viride* A7	50.63 (-28.00)
7	*T. harzianum* D20	42.52 (-39.85)
8	*T. harzianum* D17	43.85 (-37.42)
9	*T. harzianum* D6	44.23 (-36.85)
10	*T. harzianum* D2	47.37 (-32.42)
11	*T. harzianum* D12	49.41 (-29.42)
12	*T. harzianum* D15	52.90 (-24.42)
13	*T. harzianum* D19	50.11 (-28.42)
Control *P. aphanidermatum*		70.00
SE		1.13
CD(P=0.05)		3.47

* Figures in parentheses indicate % reduction in growth of pathogen over control

In pot culture experiments carried out in green house *Trichoderma sp*. were evaluated for the control of pre-emergence seed rot and post emergence damping off. Based on the activity of *Trichoderma sp*. on inhibition of growth of *P. aphanidermatum* in dual culture, five isolates were selected whose zone of inhibition was 12 mm or more. Thus three isolates of *T. viride* and two isolates of *T. harzianum* were selected in pot culture experiments.

Experiments were conducted in randomized block design. Initially isolates were evaluated for method of application employing two different methods viz. soil and seed application. In the later part of work, five selected isolates of *Trichoderma* sp. were studied for their field efficiency in controlling pre-emergence seed rot and post emergence damping off of chilli where isolates were applied as soil amendment method.

Among the thirteen isolates tested the five best isolates were used in further work. Isolates of *Trichoderma* sp. were grown on the PDA and distilled water spore suspension was used as inoculum. Seeds of the chilli variety Jwala were coated with *Trichoderma sp.* (10^5 conidia / ml) using 0.5% carboxymethyl cellulose (CMC) as adjuvant separately. Chilli seeds treated with Thiram at 4 g / kg were used for comparison and seeds treated with 0.5% CMC only served as control. For

soil amendment of *Trichoderma,* conidial suspension made from 15-day-old culture grown on PDA was mixed in the top layer of soil at 5g kg $^{-1}$ before planting.
P. aphanidermatum isolated from diseased sample was cultured on PDA and the spore suspension was used to make the soil infested with the pathogen. The spore suspension culture was added to a mixture of soil, FYM and sand mixed equal proportion. *P. aphanidermatum* infested soil was filled to two third portion of 20-cm diameter pots.
Ten seeds of chilli were sown in each pot and three replications were maintained for each treatment and the experiment was repeated twice. The pots were observed for pre-emergence seed rotting at 7 days after sowing, and for damping off after 21 days after sowing. The pots were watered as and when required.
Isolates *Trichoderma* spp. were effective in reducing the pre-emergence seed rot as well as damping off when applied as soil amendment as well as seed treatment compared with control. Percent appearance of pre emergence seed rot and damping off was less in soil amendment as compared with seed application (Table 3). All the selected isolates were at par with seed treatment of Thiram and significantly higher over control in reducing the disease percent. Among the five strains selected, *T. viride* A14 was found to be significantly superior over other isolates for both pre emergence seed rot and damping off. However, the superior among the isolates *T. viride* A14 was closely followed by *T. harzianum* D 17. In seed application least appearance of disease was observed with Thiram seed treatment, which was significantly superior over all other treatments.

Table 3: Effect of different isolates of *Trichoderma sp* on control of *P. aphanidermatum*

Sr.No.	Isolates	Soil amendment		Seed treatment	
		Pre emergence seed rot %	Damping off appearance %	Pre emergence seed rot %	Damping off appearance %
1	*T.viride* A11	4.81	6.30	8.22	13.33
2	*T. viride* A14	3.75	4.92	7.51	11.57
3	*T. viride* A10	5.20	7.55	9.83	15.45
4	*T. harzianum* D17	4.62	6.16	8.57	13.24
5	*T. harzianum* D6	6.34	8.93	10.30	17.46
6	Thiram seed treatment	3.10	4.30	3.10	4.30
control		40.50	51.68	40.50	51.68
S.E		0.37	0.42	0.45	0.51
CD@ 5%		1.18	1.29	1.34	1.55

As the isolate *T. viride* A14 was found to be significantly superior over other isolates it was used in further experiments to compare the level of significance among seed application and soil amendment. Soil amendment of *T. viride* A14 was found to be par with Thiram seed treatment. Soil amendment of *T. viride* A14 was found to be significantly superior over control (Table 4). The effectiveness of *T. viride* A 14 to damping off under field condition is currently being investigated.

Table 4: Control of *P. aphanideramatum* by *Trichoderma viride* A14

Sr.No	Treatment	Pre emergence seed rot %	Damping off appearance %
1	Seed application	7.32	11.42
1	Soil amendment	4.10	6.15
2	Thiram seed treatment	3.52	4.92
Control		37.83	49.40
S.E		0.36	0.48
CD@ 5%		1.25	1.40

The major challenge in successful; application of a biocontrol agent is to explain the biocontrol in ecological context. A better understanding of the interactions that enhance or detract the biocontrol agent will determine the long-term success of biocontrol method. Considering the results, the best method for selecting a prospective biological control agent can be a native

Trichoderma species. So in this work the isolates of *Trichoderma* species were all isolated from the various fields in the region of the appearance of the disease. The further screening of isolates in vitro lead to selection of five isolates from thirteen isolate. Howell, (2003) proposed that a candidate biocontrol agent of *Trichoderma* species can be from areas of the plant and soil where it is expected to function in disease control, and where it is growing under conditions of temperature, moisture, and nutrient availability that approximate those found in nature. As many mechanisms are involved in the biological control of plant diseases by *Trichoderma* species occur in nature may not be true *in vitro*. So the most appropriate method for screening such agent will be following the procedure involving treatment of the seed, soil, or plant with the biocontrol agent (Harman *et al.*, 2004). Experiments on this lines suggested that the suitable method of application was soil amendment rather than seed treatment. This was followed by cultivating plant in a pathogen-infested environment till the expression of the disease symptoms, where the expression of the disease incidence was measured of the disease control efficacy of the biocontrol agent.

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