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In vitro Efficacy of Biocontrol Agents against *Pythium aphanidermatum* (Edson) Fitz

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A B S T R A C T

Present experiment was conducted to evaluate the antifungal efficacy of different biocontrol agents against *Pythium aphanidermatum*. The six isolates of *Trichoderma viz.*, *Trichoderma* isolate1, *Trichoderma* isolate 2, *Trichoderma* isolate 3, *Trichoderma* isolate 4, *Trichoderma* isolate 5 and *Trichoderma* isolate 6 were studied for their antagonistic nature against *P. aphanidermatum in vitro*using dual culture method. It was observed that all the *Trichoderma* isolates acted as antagonists and significantly reduced the mycelial growth of the pathogen (3.10 to 4.6 cm) over control (9.00 cm).

Introduction

*Pythium*spp. are commonly referred to as water moulds that belongs to the kingdom *Chromista*, phylum *Oomycota*, class oomycetes, subclass Peronosporomycetidae and family *Pythiaceae* (Kirk *et al*., 2008). They naturally exist in soil and water as saprophytes and infect the hypocotyl of seedlings where they live as parasites. Among the *Pythium* spp. *Pythium aphanidermatum* (Edson) Fitz. iscosmopolitan in distribution and very common causing damping off in nurseries which is a major constraint in tomato production causing 62 per cent mortality of seedlings (Ramamoorthy *et al*., 2002).

The most common method to check damping off in nursery beds is the use of fungicides, but frequent and indiscriminate uses of fungicides often leads to environmental pollution and development of resistance in pathogens (Shanmugam and Varma, 1999).

Materials and Methods

Six isolates of *Trichoderma* were isolated from the rhizosphere soils of tomato seedlings.

The antagonistic potential of the biocontrol agents was tested against *P. aphanidermatum* by using dual culture technique (Dennis and Webster, 1971).

Isolation of the pathogen

The fungus *P. aphanidermatum* was isolated from the collar region of the infected plants showing damping-off symptoms using tissue segment method and purified by single hyphal tip method (Rangaswami, 1958). Small diseased tissues from infected collar region (3 mm) along with some healthy tissue were cut with sterile scalpel. The samples were surface sterilized with 0.1 % mercuric chloride solution for 30 sec. The diseased samples were subsequently washed in three changes of sterile distilled water to eliminate mercuric ions. The surface sterilized plant samples were transferred onto PDA medium in Petri dishes and incubated at 25 ± 1°C in inverted position to avoid contamination and growth was observed periodically.

Isolation of fungal biocontrol agents

Fungal antagonists were isolated by following serial dilution technique (Johnson and Curl, 1977). Soil samples were collected from the rhizosphere of tomato seedlings and were shade dried prior to use. 1 g of soil was suspended in 9ml of sterile water blank to make a suspension. Serial dilutions 10^{-2} to 10^{-7} were made by pipetting 1ml into additional 9 ml water blanks. Finally 1 ml aliquot of desired dilution was added to molten and lukewarm medium and poured into sterilized Petri plates. Plates were rotated gently on the laminar air flow bench to get uniform distribution of soil suspension in the medium. Then the plates were incubated in an inverted position at 25 ± 1ºC and observed for desired colonies. For enumeration of fungi, 10^{-3} dilution of spore suspension was taken for isolation. Isolation of fungal antagonists was specifically done on Trichoderma selective medium with three day old colonies of fungal antagonists *i.e., Trichoderma* which were picked up and purified by single spore method.

All the treatments were replicated four times and incubated at 25±1^{0}C. Observations were recorded pertaining to colony diameter and the per cent inhibition of the pathogen over control was calculated by adopting the formula as suggested by Vincent (1927).

$$\text{Per cent inhibition (I \%)} = \frac{C - T}{C} \times 100$$

I = Per cent inhibition in mycelial growth
C = Growth of pathogen in control plates
T = Growth of pathogen in treatment plates

Dual culture technique

Twenty ml of autoclaved PDA was poured aseptically in to 9 cm sterile Petri plates and 2 mm mycelial discs were cut from the edge of actively growing three day old culture of the pathogen and of the fungal antagonist with the help of sterilized cork borer and placed at the periphery at about 1 cm from the edge of the Petri plate opposite to one another. Pathogen without antagonist served as control.

Results and Discussion

In vitro efficacy of *Trichoderma* isolates on *P. aphanidermatum*

The six isolates of *Trichoderma viz., Trichoderma* isolate 1, *Trichoderma* isolate 2, *Trichoderma* isolate 3, *Trichoderma* isolate 4, *Trichoderma* isolate 5 and *Trichoderma* isolate6 were studied for their antagonistic nature against *P. aphanidermatum in vitro* using dual culture method.

It was observed that all the *Trichoderma* isolates acted as antagonists and significantly reduced the mycelial growth of the pathogen (3.10 to 4.6 cm) over control (9.00 cm) (Table 1 and Plate 1) (Fig. 1-3).

Among the different isolates, isolate 3 was found significantly superior over other isolates with the highest inhibition per cent (65.56 %) with a minimum radial growth (3.35 cm) and was followed by isolate 6 (61.67) and isolate 1 (60.00) which were on par with each other. Isolate 2 was slow growing and showed least inhibition of test pathogen *Pythium* which had overgrown the *Trichoderma*, though isolate 4 was fast in in its mycelial growth but it failed to inhibit pathogen and was overgrown by the *Pythium*. Rest of the isolates had grown fast and showed lysis at the point of interaction and inhibited the growth of pathogen mycelium (Fig. 3).

The present results are in accordance with reports of Rattan *et al.*, (2017) who reported inhibition of *Pythium* spp. by *T. koningii* (51.13%), *T. harzianum* (47.8%) and*T. longibrachiatum* (35.56%), *T. Viride* and *T. hamatum* (44.94%).

Table.1 Efficacy of fungal antagonists against *P. Aphanidermatum*

S.No.	*Trichoderma* as fungal biocontrol agent	*Mean Radial Growth (cm)	Inhibition (%)
1	Isolate1	3.60	60.00
2	Isolate2	4.60	48.89
3	Isolate3	3.10	65.56
4	Isolate4	3.92	56.39
5	Isolate5	3.62	59.72
6	Isolate6	3.45	61.67
7	Control	9.00	-
	SEm±	**0.062**	
	C.D. (0.05%)	**0.184**	-
	CV%	**2.782**	-

*Mean of four replications

Fig.1 Lysis of pathogen Mycelium at the point of interaction; **Fig.2** Microbial interactions of *Trichoderma* with *P. aphanidermatum*

Fig.1

Fig. 2

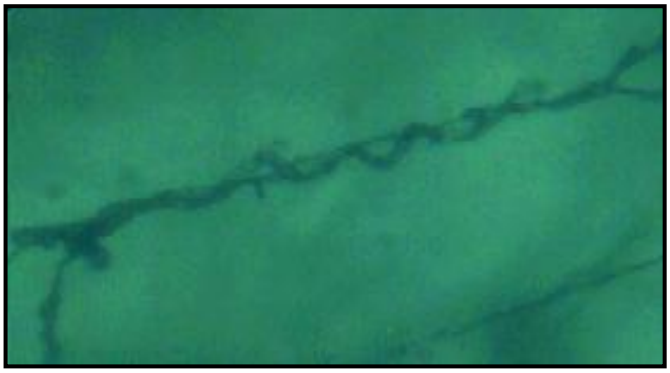


Fig.3 Inhibition in the growth of *P. aphanidermatum* dual cultured with *Tricoderma* strains

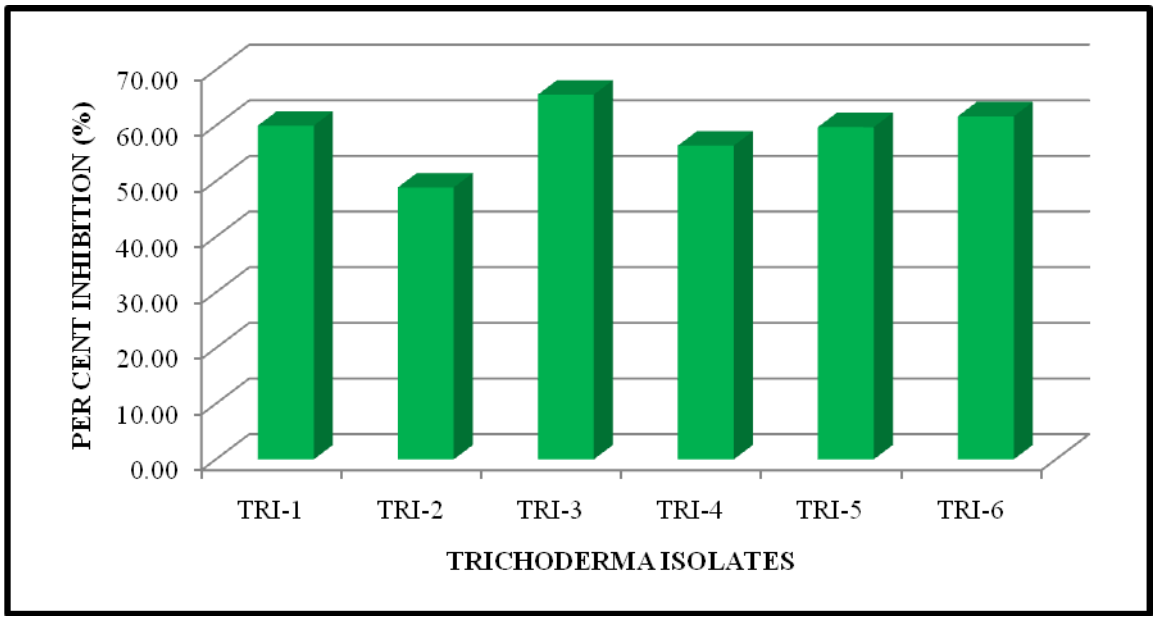
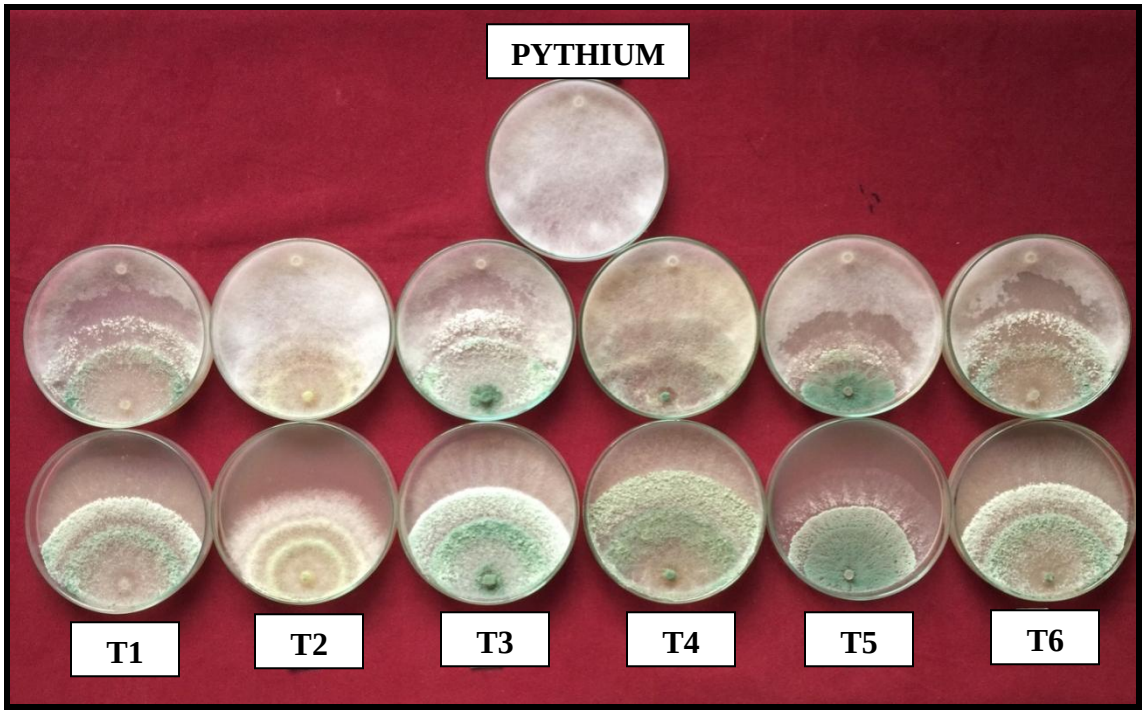


Plate.1 *In vitro* efficacy of *Trichoderma* isolates against *P. aphanidermatum* by dual cultue method



Thakur *et al.*, (2017) also reportedfungal inhibition by *T. Harzianum* (50.28%) and *T. hamatum* (44.94%) over *Pythium* and *Fusarium* sp. causing ginger rhizome rot. *In*

vitro experiments conducted by Muthukumar *et al.,* (2011) with eight *Trichoderma* isolates from chilli rhizosphere showed that TVC_3 isolate recorded maximum growth inhibition on mycelial growth of *P. aphanidermatum* (88.0%) compared with control, followed by THC_1 (83.9%) and TVC_5 (80.0%).

Muthukumar *et al.,* (2010) studies also reported highest mycelial growth inhibition of *P. Aphanidermatum* with the combination of *T. viride* +*P. fluorescens* + Zimmu leaf extract.

Microbial interactions of *Trichoderma*isolate 3 also showed antagonistic with the pathogen (Fig. 2).

Neelamegan (2004) also evaluated *Trichoderma* isolates *viz.T. viride*, *T. harzianum* and *Laetisaria* for the antagonistic activity against *P. indicumin vitro*.Among them, *T. viride* was highly inhibitory to *P. indicum*. Volatile andnon-volatile antibiotics of *T. viride* significantly inhibited the growth of *P. indicum in vitro*.

Ten strains of *Trichoderma* species were screened against *P. aphanidermatum* by dual culture method. Efficacy of culture filtrates ofthe strains was also determined.

Since mycoparasitism plays importantrole in antagonism mechanism of *Trichoderma* spp., extracellularenzymatic activity of the strains was assayed. Among the strains tested, *T. viride* 1433 was found most effective against *P. aphanidermatum* (Vinit Kumar Mishra, 2010).

In conclusion, it was observed that among the different isolates tested, isolate 3 was found significantly superior over other isolates with the highest inhibition per cent (65.56 %) and with a minimum radial growth.

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