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EFFECT OF CULTURE FILTRATE OF ISOLATES OF *TRICHODERMA* SPECIES ON MYCELIAL GROWTH INHIBITION OF FUNGI CAUSING ROT OF TOMATO AND BRINJAL

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ABSTRACT

The present study was carried out to know the efficacy of culture filtrates of *Trichoderma* species in managing the rot causing fungi of tomato and brinjal under *in vitro* conditions. It was observed from the study that all the concentrations of culture filtrates brought about significant inhibition in mycelial growth of all rot causing fungi. However, highest inhibition was observed at higher concentrations followed by lower concentrations of culture filtrates of antagonists. It was revealed from the present study that all the concentrations of isolates of *Trichoderma* species showed antimycotic activity against rot causing fungi. Culture filtrate of all the three isolates of *Trichoderma* species showed maximum activity against *Aspergillus niger* at highest concentration followed by *Penicillium expansum*, *Mucor plumbeus* and least activity was shown against *Alternaria alternata* respectively. The inhibition in mycelial growth increased with the increase in the concentration of culture filtrate of isolates of *Trichoderma*. It was further revealed from the results that the culture filtrate of *Trichoderma harzianum* isolate (PPT1) showed maximum inhibition in mycelial growth against *Trichothecium roseum* and least inhibition against *Rhizoctonia solani* at higher concentrations of culture filtrate. However, culture filtrate of *Trichoderma viride* isolate (PPT2) showed maximum mycelial growth inhibition against *Rhizoctonia solani* and against *Penicillium chrysogenum* and least inhibition against *Trichothecium roseum* at highest concentration respectively. Likewise, culture filtrate of *Trichoderma virens* isolate (PPT3) showed maximum inhibition against *Rhizoctonia solani* at higher concentration of culture filtrate of *Trichoderma* and least inhibition against *Penicillium chrysogenum* at the same concentrations respectively.

KEYWORDS: Biocontrol agents, culture filtrate, rot causing fungi, tomato and brinjal.

1. INTRODUCTION

Vegetables constitute important components of human diet. They are important sources of carbohydrates, minerals and vitamins. Vegetables are attacked by many pathogens like fungi and bacteria and result in loss of fresh produce (Mitcham and Mitchell, 2002). Amongst the vegetables, tomato and brinjal are considered important vegetables throughout the world. Tomato (*Lycopersicon esculentum* Mill.) belongs to family Solanaceae and is widely grown vegetable in the world. Rot diseases by fungi and bacteria caused heavy losses to the vegetables in storage as well as in fields (Wani and Shah, 1998; Snowdon, 2003). Brinjal (*Solanum melongena* L.) belongs to family Solanaceae and is an indigenous vegetable crop of India. It contributes 9% of the total vegetable production of the country. Postharvest decay of fruits and vegetables can be attributed to infections that occur either between flowering and fruit maturity or during harvesting and subsequent handling and storage (Droby, 2006). Therefore, aim of the present study was to evaluate the efficacy of culture filtrates in management of rot causing fungi of tomato and brinjal.

2. MATERIALS AND METHODS

2.1. Test organisms

The test fungal organisms used in this study (*Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani*) were obtained from Section of Mycology and Plant Pathology, Department of Botany, University of Kashmir, Srinagar.

2.2. Effect of culture filtrates of local isolates of *Trichoderma* spp. on the mycelial growth of fungi causing rot of tomato and brinjal

The effect of culture filtrates produced by *Trichoderma* isolates on postharvest rot fungi was studied following the method given by Dennis and Webster (1971).

Trichoderma isolates were inoculated in 100 ml sterilized Richards solution in 250 ml conical flasks and incubated at 25±2°C in an incubator for 15 days. The mycelium was collected after 15 days. The culture filtrate was filtered through Whattman filter paper and then centrifuged at 10,000 rpm for 20 min to obtain cell free culture filtrate (El-Boghdady, 1993). Appropriate volumes of the filtrates were added to the molten PDA medium to obtain final concentrations 5%, 10%, 15% and 20%. The medium placed in the petriplates was inoculated with mycelial disc of the pathogen colony from the 7 days old culture. The plates were then incubated at 25±2°C for 5 days. There were three replicates for each treatment including the control. The percentage of inhibition of mycelial growth of fruit rot fungal pathogens by culture filtrate produced by *Trichoderma* spp. in treatments was calculated on the basis of the difference with the fungal colony diameter in control plates.

$$\text{Inhibition \%} = \frac{\text{Mycelial diameter in control} - \text{Mycelial diameter in treatment}}{\text{Mycelial diameter in control}} \times 100$$

2.3. Statistical analysis of the data

The data collected during these investigations were subjected to appropriate statistical analysis using SPSS statistical software (version 16).The data was statistically analyzed by one way analysis of variance (ANOVA) and comparison of the means was done by Duncan multiple comparison tests at $P \leq 0.05$. Standard deviation was calculated as

$$\delta = \sqrt{\frac{\sum x^2}{N-1}}$$

3. RESULTS

3.1. Effect of culture filtrate of isolates of *Trichoderma* species on mycelial growth inhibition of fungi causing rot of tomato

The culture filtrates of three isolates of *Trichoderma* spp. viz., *Trichoderma harzanium* (PPT1), *Trichoderma viride* (PPT2) and *Trichoderma virens* (PPT3) at different concentrations were evaluated for their antagonistic activity against pathogenic fungi such as *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata* and *Mucor plumbeus.* It was revealed from the present study (Table 1, Fig 1) that all the concentrations of isolates of *Trichoderma* species showed antimycotic activity against tested pathogenic fungi. Culture filtrate of all the three isolates of *Trichoderma* species showed maximum activity against *Aspergillus niger* at highest concentration followed by *Penicillium expansum, Mucor plumbeus* and least activity was shown against *Alternaria alternata* respectively. The inhibition in mycelial growth increased with the increase in the concentration of culture filtrate of isolates of *Trichoderma*. However, 20% concentrations in all the three culture filtrates proved more effective than 15%, 10% and 05% concentrations. It was observed from the results that culture filtrate of isolate of *Trichoderma* (PPT3) inhibited the mycelial growth of *Penicillium expansum* to an extent of 45.65% at 20%, 37.69% (15%), 30.43 % (10%) and 28.26 % (05%) respectively followed by culture filtrate of *T. viride* (PPT2) where inhibition in mycelial growth was 41.30%, 32.60%, 26.82% and 23.91% respectively. The least inhibition in mycelial growth was found in culture filtrate of isolate *T. harzianum* (PPT1), In this case the inhibition in mycelial growth was found as 34.78%, 26.08%, 23.91% and 18.13% at 20%, 15%, 10% and 5% concentrations of *Trichoderma* isolates. Likewise, in case of *Aspergillus niger*, *Alternaria alternata* and *Mucor plumbeus* highest inhibition in mycelial growth was found at the same concentrations of isolate *T. virens* (PPT3) followed by isolate *T. viride* (PPT2) and isolate *T. harzianum* (PPT1) respectively.

Table 1: Effect of culture filtrate of isolate of *Trichoderma* species on percent growth inhibition of rot causing fungi of tomato under *in vitro* condition.

Treatment \ Concentration		Mycelial growth (mm)			
		Penicillium expansum	***Aspergillus niger***	***Alternaria alternata***	***Mucor plumbeus***
Trichoderma harzanium (PPT1)	5%	37.66±1.52^{b} (18.13)	50.33±1.52^{b} (11.17)	35.00±2.00^{b} (15.98)	37.00±1.00^{b} (17.15)
	10%	35.00±1.00^{c} (23.91)	46.00±1.00^{c} (18.81)	33.33±1.52^{bc} (19.99)	34.33±0.57^{c} (23.13)
	15%	34.00±1.00^{cd} (26.08)	35.00±1.00^{e} (38.22)	32.00±1.00^{cd} (23.18)	33.00±1.00^{cd} (26.10)
	20%	30.00±1.00^{ef} (34.78)	32.66±1.52^{f} (42.35)	30.00±1.00^{def} (27.98)	30.66±0.57^{e} (31.34)
Trichoderma viride (PPT2)	5%	35.00±1.00^{c} (23.91)	41.00±1.00^{d} (27.63)	35.00±1.00^{b} (15.98)	34.66±0.57^{c} (22.39)
	10%	33.66±1.52^{cd} (26.82)	35.00±1.00^{e} (38.22)	32.00±1.00^{cd} (23.18)	31.00±1.52^{e} (30.58)
	15%	31.00±1.00^{de} (32.60)	32.00±1.00^{f} (43.52)	29.00±2.00^{ef} (30.38)	30.66±1.52^{e} (31.34)
	20%	27.00±1.00^{g} (41.30)	31.00±1.00^{fg} (45.28)	28.00±1.00^{f} (32.78)	27.33±0.57^{f} (38.80)
Trichoderma virens (PPT3)	5%	33.00±1.00^{cd} (28.26)	40.00±1.00^{d} (29.40)	34.66±1.15^{b} (16.80)	31.66±1.52^{de} (29.10)
	10%	32.00±1.00^{cde} (30.43)	32.66±1.52^{f} (42.35)	30.66±0.52^{de} (26.40)	31.00±1.00^{e} (30.58)
	15%	28.66±1.52^{fg} (37.69)	31.66±1.52^{f} (44.12)	30.33±0.57^{def} (27.19)	27.66±0.57^{f} (38.06)
	20%	25.00±1.00^{h} (45.65)	29.33±1.52^{h} (48.23)	28.00±1.00^{f} (32.78)	25.00±1.00^{g} (44.02)
Control		46 mm ±1.00^{a}	56.66mm ±1.52^{a}	41.66 mm ±1.52^{a}	44.66 mm ±1.52^{a}

Each value is mean of 3 replicates ±SD
Figures in parenthesis is the mycelial growth inhibition (%)

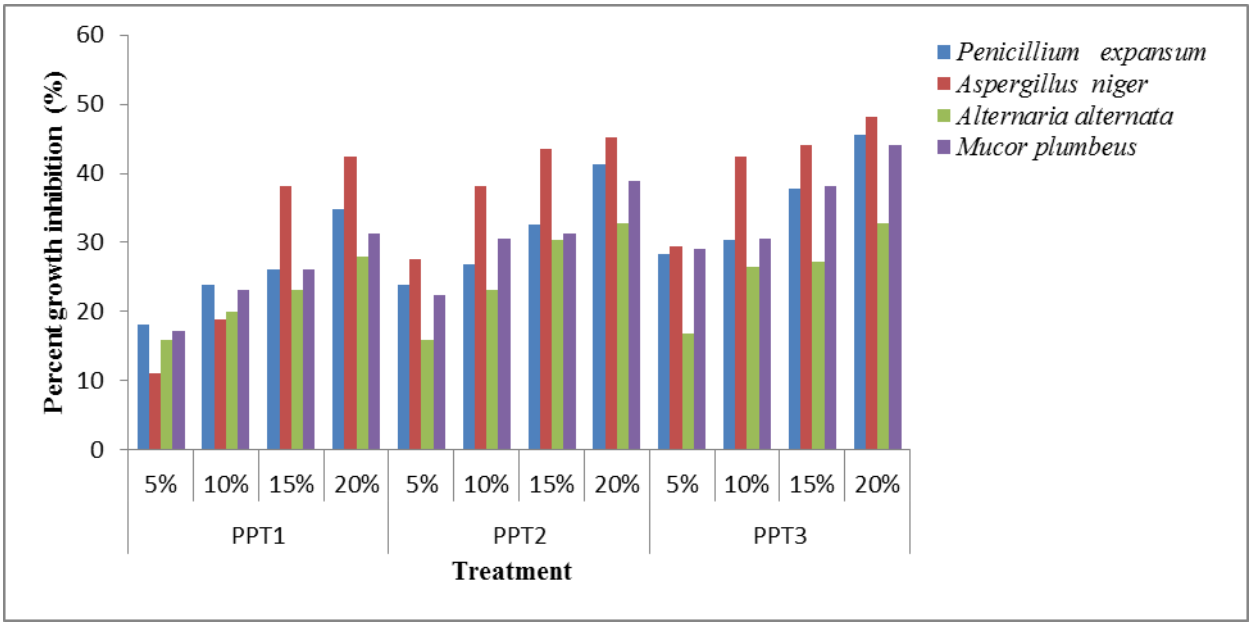

Fig 1. Effect of culture filtrate of isolates of *Trichoderma* species on the percent growth inhibition of rot causing fungi of tomato under *in vitro* conditions.

3.2. Effect of culture filtrate of isolates of *Trichoderma* species on the mycelial growth inhibition of fungi causing rot of brinjal

It was observed from the results (Table 2, Fig 2) that culture filtrate of isolates of *Trichoderma* species at different concentrations brought about significant inhibition in the mycelial growth compared to control. The culture filtrate of *Trichoderma harzianum* isolate (PPT1) showed maximum inhibition in mycelial growth against *Trichothecium roseum* and least inhibition against *Rhizoctonia solani* at higher concentrations of culture filtrate. However, culture filtrate of *Trichoderma viride* isolate (PPT2) showed maximum mycelial growth inhibition against *Rhizoctonia solani* and against *Penicillium chrysogenum* and least inhibition against *Trichothecium roseum* at highest concentration respectively. Likewise, culture filtrate of *Trichoderma virens* isolate (PPT3) showed maximum inhibition against *Rhizoctonia solani* at higher concentration of culture filtrate of *Trichoderma* and least inhibition against *Penicillium chrysogenum* at the same concentrations respectively. It was further observed from the results that culture filtrate of isolate *Trichoderma harzianum* (PPT1) inhibited the mycelial growth of *Penicillium chrysogenum* to an extent of 37.73%, 33.34%, 26.31% and 18.42% at 20%, 15%,10%, 05%, concentrations followed by culture filtrate of *T. viride* isolate (PPT2) and culture filtrate of *T. virens* isolate (PPT3) respectively. Likewise, culture filtrate of *T. harzianum* isolate (PPT1) caused highest inhibition in mycelial growth against *Trichothecium roseum* and *Rhizoctonia solani* and it was followed by culture filtrate of *T. viride* isolate (PPT2) and by culture filtrate of *T. virens* isolate (PPT3) respectively.

Table 2: Effect of culture filtrate of isolates of *Trichoderma* species on the percent growth inhibition of rot causing fungi of brinjal under *in vitro* condition.

Treatment \ Concentration		Mycelial growth (mm)		
		Penicillium chrysogenum	***Trichothecium roseum***	***Rhizoctonia solani***
Trichoderma harzianum (PPT1	5%	31.00±1.00^{cde} (18.42)	31.66±1.15^{def} (19.50)	32.00±1.00^{d} (20.00)
	10%	28.00±1.00^{fg} (26.31)	30.33±1.52^{efg} (22.88)	31.00±1.00^{de} (22.50)
	15%	25.33±1.15^{hi} (33.34)	26.66±0.57^{h} (32.21)	27.33±1.52^{f} (31.67)
	20%	23.66±0.57^{i} (37.73)	23.66±0.57^{i} (39.84)	25.66±1.52^{f} (35.85)
Trichoderma viride (PPT2)	5%	33.66±1.15^{b} (11.42)	34.00±1.00^{c} (13.55)	34.00±1.00^{c} (15.00)
	10%	30.66±1.15^{de} (19.31)	31.33±1.15^{efg} (20.34)	32.66±0.57$^{c\ d}$ (18.35)
	15%	28.33±0.57^{e} (25.44)	29.66±1.15^{g} (24.58)	29.66±1.52^{e} (25.85)
	20%	26.33±0.57^{gh} (30.71)	27.33±0.57^{h} (30.51)	27.33±0.57^{f} (31.67)
Trichoderma virens (PPT3)	5%	33.00±1.00^{bc} (13.15)	36.00±1.00^{b} (8.46)	36.66±0.57^{b} (8.35)
	10%	32.33±1.52^{bcd} (14.92)	33.33±0.57^{cd} (15.25)	34.33±0.57^{c} (14.17)
	15%	31.66±1.52^{bcd} (16.68)	32.00±1.00^{de} (18.63)	31.00±1.00^{de} (22.50)
	20%	29.33±1.52^{ef} (22.81)	30.00±1.00^{fg} (23.72)	29.33±1.15^{e} (26.67)
Control		38.00 mm ±1.00^{a}	39.33 mm ±1.52^{a}	40.00 mm ±1.00^{a}

Each value is mean of 3 replicates ± SD

Figures in parenthesis is the mycelial growth inhibition (%)

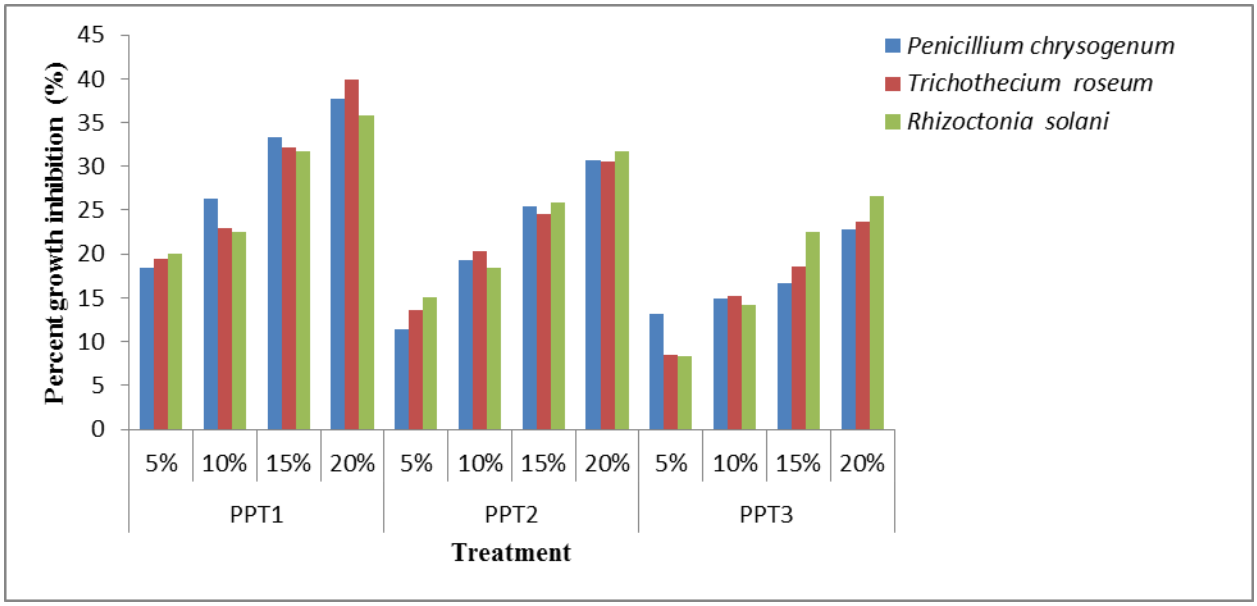

Fig. 2: Effect of culture filtrate of isolates of *Trichoderma* species on the percent growth inhibition of rot causing fungi of brinjal under *in vitro* conditions.

DISCUSSION

It was observed from the study that all the concentrations of culture filtrates brought about significant inhibition in mycelial growth of all rot causing fungi. However, highest inhibition was observed at higher concentrations followed by lower concentrations of culture filtrates of antagonists. Odebode (2006) tested antagonistic activity of culture filtrates from *Trichoderma harzianum* Rifai and *Trichoderma pseudo-koningii* Rifai strains against post-harvest pathogens of some fruits under *in vitro* conditions. The undiluted culture filtrates of the two *Trichoderma* species completely inhibit germination of conidia/spores of all the rot pathogens and percentage inhibition decreases by diluting the culture filtrates. *T. pseudo - koningii* culture filtrate strongly inhibits the mycelia growth of the pathogenic fungi with highest per cent inhibition of about 45.6% of mycelial growth in *Aspergillus niger* Van Tiegh. Rajendiran *et al*. (2010) reported maximum per cent growth inhibition of *A. niger*, *A. fumigatus* and *A. flavus* in 50 per cent culture of *Trichoderma viride*. Siameto *et al*. (2010) reported that culture filtrates of *Trichoderma* species obtained from Czapek's media produced higher reduction of mycelial dry weight of soil borne pathogens compared to culture filtrates from potato dextrose broth. Similarly, Naher *et al*. (2012) also reported variations between antagonistic activity of *Trichoderma* species grown on Potato sucrose Agar (PSA) and those grown on Potato dextrose agar (PDA). Nirupama and Singh (2011) screened culture filtrate of ten *Trichoderma* isolates in order to evaluate the production of volatile and nonvolatile inhibitors against the *Fusarium oxysporium.* Naresh (2014) reported that culture filtrate of *Trichoderma viride* and *Trichoderma harzianum* was effective in inhibiting the mycelial growth of *F. solani*. Alka and Prajapati (2017) studied the effect of culture filtrate of six *Trichoderma* spp, viz. *T. viride*, *T. harzianum*, *T. virens*, *T. atroviridae*, *T. fasciculatum* and *T. asperellum* against *Rhizopus oryzae* by poisoned food technique and found that *T. asperellum* and *T. viride* proved more effective and least inhibition was due to *T. virens.*

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