Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 11 [11] October 2022 :215-218

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Effect of volatile and non-volatile metabolites of *Trichoderma asperellum* isolates on thegrowth of *Pythium aphanidermatum* in Tomato

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ABSTRACT

The main aim of this study was to find out whether Trichoderma asperellum spp. havethe potential as a bio-control agent against damping off of tomato which is caused by pythium aphanidermatum. Damping off is a major nursery disease of tomato . Under in-vitro conditionsthe effect of seven Trichoderma asperellum isolates was observed against pythium aphanidermatum. All the seven Trichoderma asperellum isolates showed varied antagonistic effects against the test pathogen. Among all the isolates isolate T-13recorded maximum growth inhibition of test pathogen due the production of high quantities of volatile and non volatile metabolites. The culture filtrate of the T-13 Trichoderma asperellum isolate recorded maximuminhibition on the mycelial growth of the pathogen at 10% culture filtrate.

Keywords: Tomato*, Trichoderma asperellum,* Bio-control, *Pythium aphanidermatum, volatile ,non volatile.*

Received 22.10.2022 Revised 25.10.2022 Accepted 10.11.2022

INTRODUCTION

Tomato is an important vegetable crop in India which is attacked by several diseases which are caused by fungi, bacteria and viruses. Among fungal diseases, the damping off disease caused by *Pythium aphanidermatum* in nurseries is a major constraint in the production of Tomato. *Pythium* spp. are completely soil borne [1, 2] and create a greater problemin disease management as it is morphologically polymorphic, physiologically unique and ecologically versatile. Different species of *pythium* are considered as important and destructive plant pathogens causing losses of seeds, pre-emergence and post-emergence damping off, rots of seedlings, root rot, roots or Basal stalks, decay of fruits for many plants across the world. Previous work in this area revealed the pathogenicity of *Pythium aphanidermatum*. Despite many efforts of eradication and sanitization *Pythium aphanidermatum* persist in the different regions of Uttar Pradesh and other areas of India.

The most common method to stop the disease incidence in nurseries is use of fungicides, but the continuous and indiscriminate use of fungicides proceeded into atmospheric pollution and development of fungicide resistance in plants [3]. In the present scenario the biological control is coming up as a powerful tool because it can work continuously as long as bio-control agent is alive and active.

MATERIAL AND METHODS

Isolation of *Trichoderma* from rhizosphere soil sample

The soil samples were collected from the different locations of Kanpur, Uttar Pradesh, India at a depth ranging 5.0-6.0 cm, by removing top 2.0 cm surface soil. Isolation was done by serial dilution technique. The probable colonies of *Trichoderma* were observed closely and picked up from Petri plates and transferred to Potato Dextrose Agar (PDA) slants and finally pure culture was obtained by repeated subculture. The probable colonies of *Trichoderma* were identified upto species level on the basis of their morphological and molecular characters [4, 5].

Evaluation of antagonistic activity through production of antifungal volatile metabolites

Productions of volatile metabolites by *Trichoderma asperellum* were assayed as described by [6] with slight modifications. The *Trichoderma asperellum* isolates were centrally inoculated by placing 5mm disc taken from three days old cultures on the PDA plates and incubated at 28 ± 2° C for three days. The pathogen was inoculated centrally at the bottom of the PDA plate and this PDA plate was then replaced by the top of each Petri dish which was containing *Trichoderma* at the bottom. Petri dish with PDA

medium without *Trichoderma asperellum* at the lower lid and the upper lid with pathogens was maintained as control. Paraffin tape was used to seal each Petri dish pair and this pair was then incubated for four to six days. After incubation the inhibition of mycelia growth was calculated according to [7].

Table 1 Effect of volatile metabolites of *Trichoderma* isolates on the growth of *Pythiumaphanidermatum*

S. No.	Isolates	*Pythium aphanidermatum*	
		Average growth (mm)	Per cent inhibition over control
1.	T-11	30.33	39.65
2.	T-12	36.67	27.04
3.	T-13	27.67	44.95
4.	T-14	36.67	27.04
5.	T-15	35.67	29.04
6.	T-16	35.33	29.70
7.	T-17	36.33	27.71
8.	CONTROL	50.26	
	SE(d)	**1.75**	
	CD at 5%	**3.76**	

Observation of the effect of antifungal non-volatile metabolites production for the evaluation of the antagonistic activity

The ability of *Trichoderma asperellum* isolates to produce the non-volatile substances was studied by following methods as described by [8] with slight modifications. All test isolates of *Trichoderma asperellum* were grown in 100 ml sterilized Potato Dextrose Broth (PDB) for 10 days in 250 ml Erlenmeyer flasks with periodical shaking. Culture filtrate of *Trichoderma asperellum* was harvested by filtering it through Whatmann filter no. 42 filter paper into sterilized flasks. The required volume of culture filtrate was added with known volume of meltedPDA to obtain final concentration of 2.5%, 5.0%, 7.5%, 10.0% and 20.0% (v/v) culture filtrate. Young culture tube of the test pathogen was used for mycelia plug (five millimeter diameter). This mycelia plug was used for inoculation of the amended media poured into Petri dish. This amended media is then incubated at 28 ± 2°C for three days. For control the PDA plates without culture filtrate of Trichoderma were used. As mentioned earlier, according to [7], per cent inhibition of mycelial growth of pathogen was calculated and the radial mycelial growth of test pathogen was measured.

Table 2 Effect of non-volatile metabolites of *Trichoderma* isolates on the growth of *Pythium aphanidermatum*

S. No.	Isolates	*Pythium aphanidermatum*									
		2.5%		5.0%		7.5%		10.0%		20.0%	
		Average growth (mm)	Per cent inhibition over control	Average growth (mm)	Per cent inhibition over control	Average growth (mm)	Per cent inhibition over control	Average growth (mm)	Per cent inhibition over control	Average growth (mm)	Per cent inhibition over control
1.	T-11	24.67	39.09	18.67	53.91	16.67	58.85	13.67	66.26	14.00	65.43
2.	T-12	30.67	24.28	24.67	39.09	21.67	46.50	20.00	50.62	22.00	45.68
3.	T-13	24.00	40.74	17.00	58.02	14.33	64.61	12.33	69.55	12.67	68.72
4.	T-14	31.00	23.46	26.00	35.80	24.67	39.09	23.67	41.56	24.00	40.74
5.	T-15	29.67	26.75	25.00	38.27	18.67	53.91	16.67	58.85	17.33	57.20
6.	T-16	35.33	12.76	22.00	45.68	21.33	47.33	18.33	54.73	18.33	54.73
7.	T-17	30.00	25.93	23.33	42.39	24.00	40.74	21.33	47.33	21.33	47.33
8.	CONTROL	40.50		40.50		40.50		40.50		40.50	
	SE(d)	**1.42**		**1.74**		**1.85**		**1.66**		**1.75**	
	CD at 5%	**3.05**		**3.74**		**3.97**		**3.56**		**3.76**	

RESULTS AND DISCUSSION

Antagonistic effect of volatile substances against *Pythium aphanidermatum*

Seven *Trichoderma asperellum* isolates from different region of Kanpur, Uttar Pradesh, India have different levels of antagonism against test pathogen. All the *Trichoderma* isolates produced toxic volatile metabolites having significant effect in reducing the radial growth of test pathogen as shown in Table 01. Maximum growth inhibition of *Pythiumaph anidermatum*was recorded by the isolates T-13 (44.95 %) followed by T-11 (39.65 %), T-16 (29.70 %), T-15 (29.04 %), T-17 (27.71 %), jointly followed by T-14 (27.05%) and T-12(27.05%).

Different *Trichoderma spp.* produce diffusible volatile antibiotics and thus act against fungal plant pathogens under *in-vitro* conditions. Vey *et al.,* (2001) [9] observed that *Trichoderma spp.* produce a large varieties of volatile secondary metabolites such as C_2H_4, HCN, aldehydes and ketones. These volatile secondary metabolites play an important role in controlling the plant pathogens [10]. Amin *et al.,* (2010) [11] also observed the volatile activity of six isolates of *Trichoderma* spp. against seven different fungal plant pathogens.

Several workers[12-14] have also reported the effectiveness of diffusible volatile compounds by *T. viride* and *T. harzianum* under in vitro.

Antagonistic effect non -volatile substances against soil borne pathogens

All the *Trichoderma asperellum* isolates significantly inhibited the *Pythium aphanidermatum* by production of non-volatile inhibitors at 2.5%, 5.0%, 7.5%, 10% and 20% as shown in Table 02. Concentration wise details are as given below:

At 2.5 % concentration

Maximum percentage inhibition of test pathogen was exerted by isolate T-13 with 40.74 per cent followed by T-11 (39.09 %), T-15 (26.75 %), T-17 (25.93 %), T-12(24.28%), T-14(23.46%) and T-16 (12.76 %).

At 5.0 % concentration

Isolate T-13 was again found superior in growth inhibition (58.02 %) followed by isolate T-11 (53.91 %), T-16 (45.68 %), T-17(42.39 %), T-12(39.09%), T-15(38.27%) and T-14 (35.80 %). Isolate T-14 was least effective with 35.80 % inhibition.

At 7.50 % concentration

Isolate T-13 caused highest growth inhibition (64.61%) followed by T-11 (58.85 %), T-15 (53.91 %), T-16(47.33%), T-12(46.5 %) and T-17 (40.74%), where as T-14 was least effective with 39.09 % inhibition.

At 10.00 % concentration

Isolate T-13 caused highest growth inhibition (69.55 %) followed by T-11 (66.26 %), T-15 (58.85%), T-16(54.73 %), T-12(50.62 %), and T-17(47.33%) where as T-14 was least effective with 41.56 % inhibition.

At 20.00 % concentration

Isolate T-13 caused highest growth inhibition (68.72 %) followed by T-11 (65.43 %), T-15 (57.2%), T-16 (54.73%), T-17(47.33%) and T-12 (45.68 %), where as T-14 was least effective with 40.74 % inhibition.

Antagonisim of *Trichoderma* species against several pathogens were reported[15-17]. The degree of inhibition varied from one strain to another. In the present investigation, some of the *Trichoderma* isolate T13was highly efficient whereas some isolates have exhibited relatively lessinhibition of mycelial growth of test fungus. The possible reason may be due to their inherent potentiality to adapt well in introduced conditions and aggressiveness of the *Trichoderma* isolates towards certain plant pathogens[18,19].

Rao and kulkarni,[20] observed the production of volatile and non-volatile antibiotics and told that *T. harzianum* and *T. viride* were highly effective in reducing the radial growth of *S. rolfsii* by the production of these substances. Dubey and Suresh (2006) [21]reported that the production of non-volatile substances by *T. harzianum* are inhibitory to *F. o.* f. sp. *ciceri* causing chickpea wilt. Similarly *T. viride* isolate, followed by *T. harzianum* inhibited maximum mycelial growth of the *F. o.* f. sp. *ciceri* through production of volatile and non-volatile inhibitors in dual culture [22]. Waseem *et al.,* (2013) [23] found antifungal non volatile compounds extracted from the liquid culture *Trichoderma* strain SQR-T037, significantly inhibited the growth of *F. oxysporum*. f. sp. *niveum* incitant watermelon of wilt.

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CITATION OF THIS ARTICLE

L Tiwari and A Srivastava. Effect of volatile and non-volatile metabolites of *Trichoderma asperellum* isolates on the growth of *Pythium aphanidermatum* in Tomato. Bull. Env.Pharmacol. Life Sci., Vol 11 [11] October 2022 :215-218