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Research Note

Evaluation of fungal antagonists to control damping-off of tomato (*Lycopersicon esculentum* Mill.) caused by *Pythium indicum*

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ABSTRACT: ***Trichoderma viride, T. harzianum, Laetisaria arvalis*** **and other eight isolates of** ***Trichoderma*** **viride, isolated locally, were tested** ***in vitro*** **for their antagonistic activity against** ***Pythium indicum*****, the causal organism of damping-off of tomato, by dual agar technique. Among the fungal antagonists tested,** ***Trichoderma viride*** **was found highly inhibitory to** ***P. indicum*****. Volatile and non-volatile antibiotics of** ***T. viride*** **inhibited the** ***in vitro*** **growth of** ***P. indicum*** **significantly.**

KEY WORDS: Damping-off, *Pythium indicum*, *Trichoderma viride*, volatile and non-volatile antibiotics

Damping – off, a fungal disease of tomato, caused by *Pythium* species accounts for severe crop loss in all the areas where the seedlings are crowded in nursery bed, greenhouse plot and row crops. The control of *Pythium* is difficult due to its wide host range, soil born nature, and prolonged survival of propagules in soil. These constraints necessitate controlling plant diseases through biological means. In recent times, there has been considerable interest in the biocontrol of plant pathogens, particularly soil borne pathogens, by the purposeful introduction of *Trichoderma* species (Prasad *et al.*, 1999; Mukhopadhyaya *et al.*, 2001; Gnanavel and Jayaraj, 2003). In the present study, an attempt was made to select an effective fungal antagonist to control *Pythium indicum* Balakrishnan, a causal organism of damping-off of tomato (*Lycopersicum esculentum* Mill.).

The soil pathogen, *P. indicum*, was isolated from diseased tomato seedlings and identified based on characters described by Balakrishnan (1948) and its pathogenicity was established. Eight isolates of *Trichoderma* viride isolated from the rhizosphere soil of healthy tomato seedlings, collected at different locations by Double Agar Layer Technique (Freeman & Tims, 1955) and three identified fungal antagonists (i.e., *Trichoderma viride, Trichoderma harzianum* and *Laetisaria arvalis)* obtained from Tamil Nadu Agricultural University, Coimbatore were used (Table 1) to test their antagonistic activity against *P. indicum* by dual culture technique (Dennis & Webster, 1971c). The radial growth of the pathogen (*P. indicum*) and the test fungi was measured at different incubation period up to 120 hours. The width of the inhibition zone was measured and the growth character was observed. Production of volatile and non-volatile antibiotics by *T. viride* was assayed against *P. indicum* at prefixed intervals (Table 3) and were tested following the procedure of Dennis and Webster (1971a & b). The rate of antibiotic

production was estimated by the degree of inhibition of the growth of *P. indicum*. The *in vitro* studies were carried out at room temperature (28 ±2°C) with three replications.

Eleven fungal antagonists were tested to evaluate their effect on the radial growth of *P. indicum* in dual culture. It is evident from the results (Table 1) that the radial growth of *P. indicum* was adversely affected by the antagonists, i.e., *T. viride*, *T. harzianum*, *L. arvalis* and three isolates of *Trichoderma viride* (S_1A_1, S_2A_1, and S_4A_3), which were not significantly different from each other.

Trichoderma viride caused an antagonized zone of 15 mm width and showed over growth, coiling, lysis and profuse sporulation (Table 2). *T. harzianum* exhibited an antagonized zone of 12 mm width and showed coiling, lysis, and profuse sporulation. Further, it formed a relief zone (instead of over growth) as an expression of antagonism. *Trichoderma viride* isolates S_1A_1, S_2A_1 and S_4A_3 formed 1.3mm, 4mm and 4.3mm width of antagonized zone, respectively and they displayed overgrowth, lysis and profuse sporulation. *L. arvalis* formed an antagonized zone of 8.7mm width and revealed over growth, coiling and sclerotial production. Among the antagonist, *T. viride* offered the maximum antagonized zone of 15mm width in dual culture with *P. indicum*. From the results, it is evident that the antagonists isolated from the rhizosphere of tomato grown in local soil were less effective in controlling *P. indicum* than *T. viride* obtained from TNAU, Coimbatore.

Antagonistic activity of *Trichoderma* species against *Pythium* species has been reported earlier by several workers (Lifshitz *et al.*, 1984; Sivan *et al.*, 1984; Gnanavel and Jayaraj, 2003). The mechanism of antagonism expressed by *T. viride*, in this study, against *P. indicum* is over growth, coiling and lysis (mycoparasitism). It is observed that *T. viride* grew towards the hyphae of *P. indicum*

Table 1. Effect of antagonistic fungi on the radial growth (mm/72 h) of *P. indicum* in dual culture

Test fungus	Source of Test Fungus	Growth of *P. indicum*	Per cent change
Trichoderma viride (S_1A_1)	Annamalinagar	51.3 g	- 43.0
Trichoderma viride (S_1A_2)	Annamalainagar	63.7 def	- 29.2
Trichoderma viride (S_2A_1)	Sivapuri	56.3 fg	- 37.4
Trichoderma viride (S_2A_2)	Sivapuri	65.3 de	- 27.4
Trichoderma viride (S_3A_1)	Naduthittu	69.0 cd	- 23.3
Trichoderma viride (S_4A_1)	Vallampadugai	64.0 de	- 28.9
Trichoderma viride (S_4A_2)	Vallampadugai	76.7 b	- 14.8
Trichoderma viride (S_4A_3)	Vallampadugai	57.7 efg	- 35.9
Trichoderma viride	TNAU - Coimbatore	50.3 g	- 44.1
Trichoderma harzianum	TNAU - Coimbature	55.7 g	- 38.1
Laetisaria arvalis	TNAU - Coimbature	53.0 g	- 41.1
Control - *P. indicum*	–	90.0 a	–

- In a column, the means followed by similar letters are not significantly different (DMRT).

Table 2. Antagonism of fungal antagonists against *P. indicum* in dual culture

Antagonist	Mean radial growth (mm)				Width of antagonized zone (mm)	Growth characters of antagonist
	48 h		120 h			
	AN	PI	AN	PI		
T. viride	29.0	61.0 ab (-5.72)	44.0	46.0 cd (48.89)	15.0	Over growth, coiling, lysis, profuse sporulation
T. harzianum	26.0	64.0 a (-1.08)	38.0	52.0 cd (-42.22)	12.0	Coiling, lysis, profuse sporulation.
T. viride – ($S_1 A_1$)	40.7	49.3 c (-23.80)	42.0	48.0 cd (-46.67)	01.3	Over growth, profuse sporulation.
T. viride – ($S_2 A_1$)	41.0	49.0 c (-24.27)	45.0	45.0 d (-50.00)	04.0	Over growth, profuse sporulation.
T. viride – ($S_4 A_3$)	37.0	53.0 bc (-18.08)	41.3	48.7 cd (-45.89)	04.3	Over growth, profuse sporulation
L. arvalis	27.3	62.7 a (-3.09)	36.0	54.0 bc (-40.00)	08.7	Over growth, coiling, sclerotia produced.
Control	–	64.7 a (00.00)	–	90.0 a (00.00)	–	–

- AN – Antagonists, PI - *Pythium indicum*
  Figure in parentheses are growth reduction over control (%).
- In a column, the means followed by same letter are not significantly different (DMRT).

Table 3. Effect of volatile and non-volatile antibiotics of *T. viride* on the radial growth of *P. indicum*

Sl. No.	Age of *T. viride* days after inoculation	Growth of *P. indicum* (mm/72 h)
Volatile Antibiotics		
1	0	48.67 (-45.93) f
2	1	57.00 (-36.67) e
3	3	58.67 (-34.81) de
4	5	68.33 (-24.07) c
5	10	75.33 (-16.29) b
6	Control	90.00 (00.00) a
Non-volatile Antibiotics		
1	2	77.00 (-14.44) b
2	4	65.33 (-27.41) c
3	6	58.00 (-35.56) d
4	8	40.67 (-54.81) ef
5	10	38.33 (-57.41) f
6	Control	90.00 (00.00) a

In a column, the means followed by similar letters are not significantly different (DMRT).
Figures in parentheses represent percent inhibition over control.

in dual culture, and inhibited further growth of pathogen on contact. It shows antagonism by over growth on the hyphae of *P. indicum* in dual culture. The adpressed growth coiling of *T. viride* around the hyphae of *P. indicum* could involve antibiotic and enzyme production, which led to direct parasitism as well as lysis (D'Ercole *et al.*, 1984).

The volatile and non-volatile antibiotics produced by *T. viride* inhibit the growth of *P. indicum*. Maximum inhibition of *P. indicum* was noticed in 10 days old culture filtrate of *T. viride* (Table 3). The present study reveals that the toxic substance produced by antagonists were also effective for inhibiting the pathogen and physical contact between antagonists and pathogen may not be necessary for effective antibiosis (Narasimha Rao and Kulkarni, 2003). Several workers reported the inhibitory effects of both volatile and non-volatile substances produced by *Trichoderma* species on several soils born pathogens (Dennis and Webster, 1971b; Upadhyay and Mukhopadhyay, 1983; Mathur and Bhatnagar, 1994). The control of *P. indicum* by *T. viride*, in this study, was carried out through the antagonistic activities such as overgrowth coiling, lysis and production of volatile and non-volatile antibiotics.

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