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Production of lytic enzymes by *Trichoderma* strains during *in vitro* antagonism with *Sclerotium rolfsii*, the causal agent of stem rot of groundnut

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Six *Trichoderma* strains (collected from IARI, New Delhi and MTCC, Chandigarh) were tested for their ability to inhibit soil-borne pathogen of groundnut mainly *Sclerotium rolfsii* (causing stem rot on groundnut). *In vitro* percent growth inhibition of *S. rolfsii* by various *Trichoderma* strains were recorded at 5th day after inoculation (DAI). Results obtained from the antagonism study indicated that *Trichoderma viride* (NBAII Tv 23) inhibited 61% growth of phytopathogenic fungi *S. rolfsii* followed by *Trichoderma harzianum* (NBAII Th1) (55% growth inhibition of pathogen). The specific activities of cell wall degrading enzymes chitinase, β-1,3 glucanase, protease and cellulase were tested during different incubation period (48, 72 and 96 h) when *Trichoderma* spp. grew in the presence of pathogen cell wall in synthetic media. The antagonist *T. viride* (NBAII Tv 23) induced higher chitinase and protease activity. The growth inhibition of pathogen during antagonism were positively correlated with coiling pattern of antagonists at 14th day after inoculation (DAI) as well as with the induction of chitinase, β-1,3 glucanase and total phenol content. However, the amount of cellulase and polygalacturonase recorded was least in these antagonists treatment. A significant positive correlation (p = 0.01) between percentage growth inhibition of test fungus and lytic enzymes (chitinase, β-1,3-glucanase and protease) in the culture medium of antagonist treatment established a relationship to inhibit growth of fungal pathogen by increasing the levels of these enzymes. Among all the tested *Trichoderma* strains, *T. viride* (NBAII Tv 23) was found to be the best strain to be used in biological control of plant pathogen *S. rolfsii*.

Key words: Antagonism, soil-borne pathogens, lytic enzyme, *Trichoderma*, biocontrol agent.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the most important oil seed crop of India. It occupies the third place with regard to acreage and in production which provides a rich source of high-quality edible oil (45-50%), easily

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digestible protein (23-25%), minerals and vitamins (Ramjeet et al., 2015). Plant diseases cause losses of agricultural crops every year. The distribution of several phytopathogenic fungi such as *Sclerotium rolfsii*, *Pythium, Phytophthora, Rizoctonia* and *Fusarium* has spread during last few years due to changes introduced in farming, with detrimental effects on crops of economic importance. Chemical compounds such as Topsin-M, Rhizolex, Benlite and Vitavax have been used to control plant diseases but it has adverse effect that creates health hazards for humans and other non-target organisms (Graham-Bryce, 1981). The development of an alternative safer and environmentally feasible plant disease control has become a top priority. In this context, biological control has become an urgent need for modern agriculture. Fungi of the genus *Trichoderma* are potential biocontrol agents of several soil-borne phytopathogens (Howell, 2003; Hassan, 2014). *Trichoderma* strains are free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. Among *Trichoderma* species, *Trichoderma harzianum, Trichoderma viride* and *Trichoderma reesei* are known as producers of these extracellular hydrolytic enzymes. *T. harzianum* is a known producer of the extracellular hydrolytic enzyme, chitinase, which is one of the biocontrol mechanisms exerted by *Trichoderma* towards fungal pathogens, besides antibiosis and competition, which degrades the chitin polymers of the fungal cell wall (Kushwaha and Verma, 2014) Chitinase was used for biological control of fungal diseases (Prabavathy et al., 2006; Srivastava et al., 2010; Hassan, 2014). In industry, chitinase enzymes are extensively used for the degradation and other processes of cellulose materials particularly in textile and paper. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. Among the plant pathogens, *S. rolfsii* was found to be cause of the stem rot disease in groundnut (Harman et al., 2004; Ruiz-Herrera, 1992; Schirmbock et al., 1994; Kubicek et al., 2001). *Trichoderma* have been considered to play an important role in the biological control of stem rot diseases. The main objective of this study was to evaluate the interaction between the antagonists *Trichoderma* strains and fungal pathogen *S. rolfsii* and lytic enzyme assay during antagonism between tested antagonists and the plant pathogen (*S. rolfsii*).

MATERIALS AND METHODS

Dual culture test

Colony interaction between antagonistic fungi and pathogenic fungi were determined by the method described by Dennis and Webster (1971), 5 mm diameter mycelial disc from the margin of one week-old culture of individual antagonists (*Trichoderma* species) and the test pathogen *S. rolfsii* were placed on the opposite to each other in the plate at equal distance from the periphery. The experimental design used was a completely randomized with four replicates for each antagonist. In control plates (without *Trichoderma* spp.), a sterile agar disc was placed at opposite side of the inoculated disc of pathogen *S. rolfsii* and incubated at 28 ± 2°C. The growth of antagonists and test pathogen was observed on 5th and 10th day after inoculation. Index of antagonism as percent growth inhibition of *S. rolfsii* was determined by following the method of Watanabe (1984).

Plant pathogen cell wall preparation

Pathogen *S. rolfsii* was inoculated into 250 ml flasks containing 50 ml of potato dextrose broth and incubated at 28 ± 2°C for 7 days. The mycelia were then collected by filtration and washed thoroughly with autoclaved distilled water and homogenized on ice, with a homogenizer, for 5 min at the highest speed. The mycelial suspension was centrifuged at 30,000 ×*g* for 20 min at 4°C. The pellet was resuspended in distilled water and sonicated on ice 4 times for 5 min using a sonicator (Equitron-4.2L100H) (Elad et al., 1982). The suspension was centrifuged at 800 ×g for 10 min at 4°C. The pellet was collected and lyophilized. The dry cell wall of pathogen was used as substrate for the production of cell wall degrading enzymes.

Cell wall degrading enzyme production

Trichoderma spp. and *S. rolfsii* were cultured at 30°C on a synthetic medium (SM) containing grams per liter of distilled water. The ingredients of the basic medium were 1.4 g of $(NH_4)_2SO_4$, 0.2 g of KH_2PO_4, 6.9 g of NaH_2PO_4. H_2O, 0.3 g of $MgSO_4.7H_2O$, 1.0 g of peptone, 5 g glucose and 0.3 g of urea. The conidia was grown in a 250 ml broth medium at 22°C with 120 rpm shaking, and the culture supernatants were collected at 48, 72 and 96 h by centrifugation at 3000 rpm for 10 min at 4°C. These samples are used for enzyme assay. For enzyme induction in pathogen *S. rolfsii* (control), 1% of either chitin, laminarin, casein or carboxymethyl cellulose was added into liquid cultures (SM), pH adjusted to 5.5 (Marco et al., 2003). Cultures were then incubated at 30°C in a rotary shaker at 120 rpm for 96 h. After 24, 48, 72 and 96 h time interval, 5 ml of mycelium was harvested and then centrifuged at 15,000 ×g for 10 min at 4°C. The supernatant was dialyzed against distilled water and was used for enzymatic activity of chitinase, β-1,3-glucanase, protease, poly galacturonase and cellulase. The method of Folin-Lowry (Lowry et al., 1951) was used to estimate the protein content in culture supernatant and for calculating specific activity of the enzyme.

Enzyme assays

Chitinase and β-1,3 glucanase activity were assayed by colorimetric method using colloidal chitin and laminarin as substrates, respectively. The level of reducing sugar released was determined by the DNSA method, using N-acetylglucosamine and glucose as a standard (Almeida et al., 2007). Cellulase activity was determined by measuring the amount of glucose formed from carboxy methyl cellulose (Sadasivam and Manickam, 1992). Reactions were conducted for 30 min at 55°C (Collmer et al., 1988). Specific activity of chitinase, β-1,3 glucanase and cellulase were expressed as 1 µmol of reducing sugars released $h^{-1}.mg^{-1}$ protein. Non enzymatic controls were also performed using boiled enzymes and were subtracted from the enzymatic values.

Protease activity was measured using casein as a substrate. The reaction was carried out according to Charney and Tomarelli (1947). Blank was treated as zero time incubation. The amount of released total free amino acids was estimated by Ninhydrin method (Lee and Takahashi, 1966). Proteolytic activity corresponded to the

Table 1. Percent growth inhibition of *S. rolfsii* during *in vitro* antagonism with *Trichoderma* at 5th and 10th day of inoculation (DAI).

Strain no.	Treatment	Inhibition(%) at 5th DAI	Inhibition(%) at 10th DAI
T_1	*T. harzianum* NBAII Th 1 X *S. rolfsii*	55%	56.6%
T_2	*T. hamatum* NBAII Tha-1 X *S. rolfsii*	50%	50.3%
T_3	*T. koningii* MTCC 796 X *S. rolfsii*	50%	51.4%
T_4	*T. pseudokoningii* MTCC 2048 X *S. rolfsii*	44%	45%
T_5	*T. virens* NBAII Tvs 12X *S. rolfsii*	50%	52%
T_6	*T. viride* NBAII Tv 23 X *S. rolfsii*	61%	63.4%
T_7	*S. rolfsii*- Control	0	0

amount of enzyme required to cause an increase of µg free amino acids.h^{-1}.mg^{-1}protein in culture supernatant. Total phenol content was estimated as method described by Malik and Singh (1980) and calculated as µg.ml^{-1} culture supernatant using pyrocatechol as a standard.

RESULTS AND DISCUSSION

Morphological observation

Pigmentation of varying shades was recorded in some strains of *Trichoderma*. Generally, *Trichoderma* had many aerial white mycelia gradually turned to green colour. Yellow pigmentation was shown in *T. koningii* and dark green colours were observed in *T. hamatum*. *Sclerotia* begin to develop after seven to eight days of mycelial growth of *S. rolfsii*. Initially, a felty white appearance, sclerotia quickly melanize to dark brown coloration.

In vitro percent growth inhibition of pathogen *S. rolfsii*

The first apparent physical contact between *Trichoderma* spp. and its host, *S. rolfsii*, occurred within two to three days after inoculation (DAI), followed by growth inhibition. Growth inhibition of *S. rolfsii* during *in vitro* interaction with bio-control agents *Trichoderma* spp. at 5th and 10th DAI was obtained. Percent growth inhibition of pathogen (*S. rolfsii*) was higher in T6 (61%) antagonist followed by T1 (55%), T3 (50%), T2 (50%) and T4 (44%), respectively at 5th DAI. Further, mycoparasitism of antagonists were observed upto 10th DAI.

Pattern of growth inhibition of test fungus was continued with maximum increases in T6 (63.4%) followed by T1 (56.6%) antagonists during 10th DAI. Thus, it was observed that T6 antagonist (interaction between *T. viride* NBAII Tv 23 and pathogen *S.rolfsii*) have a better growth inhibition of test fungus followed by T1 (*T. harzianum* NABII Th 1×*S. rolfsii*) as compared to other *Trichoderma* spp. (Table1).

Production of cell wall degrading enzymes

Six *Trichoderma* spp., tested for antagonism with fungal pathogen, produced and secreted on induction substantial amounts of various cell wall degrading enzymes when grown in the synthetic media containing pathogen cell wall as the carbon source. There was differing patterns in cell wall degrading enzyme production by various *Trichoderma* spp. during mycoparasitism at 48, 72 and 96 h incubation with pathogen cell wall (Figures 1, 2 and 3).

Mean antagonists (T) differences on specific activity of β-1,3 glucanase was found to be significant (Tables 2 and 4). The significantly highest β-1,3 glucanase activity was recorded by *T. viride* NBAII Tv 23 (14.58 U.mg-1 protein) followed by *T. hamatum* NABII Tha 1 (14.30 U.mg^{-1} protein) antagonist. Irrespective of antagonists, mean time interval (H) showed rise in enzyme activity during 48 to 96 h when *Trichoderma* spp. grew in the presence of pathogen cell wall. Overall, the activity of β-1,3 glucanase was higher in *T. viride* NBAII Tv 23 followed by *T. hamatum* NABII Tha 1 when grown with pathogen cell wall. These corresponded to the maximum growth inhibition of pathogen *S. rolfsii* by *T. viride* NBAII Tv 23 (61%) antagonist followed by *T. hamatum* NABII Tha 1 (55%) at 5th DAI antagonism. It has been shown that β-1, 3 glucanase inhibited the growth of pathogens in synergistic cooperation with chitinase.

The chitinolytic activity induced in some antagonists comprises growth of that species. Mean antagonists (T) differences on specific activity of chitinase was found to be significant (Table 3). The significantly highest chitinase activity was recorded by *T. viride* NBAII Tv 23 (10.68 U.mg^{-1} protein) followed by *T. harzianum* NABII Th 1 (8.02 U.mg^{-1} protein) antagonist at 48, 72 and 96 h incubation. Irrespective of antagonists, mean time interval (H) showed continuous rise in enzyme activity during 48 to 96 h when *Trichoderma* spp. grew in the presence of pathogen cell wall. The interaction effect of T×H was significantly different for chitinase activity.

When expressing specific activity of protease (Table 5), antagonist mean (T) activity was found highest in *T. viride*

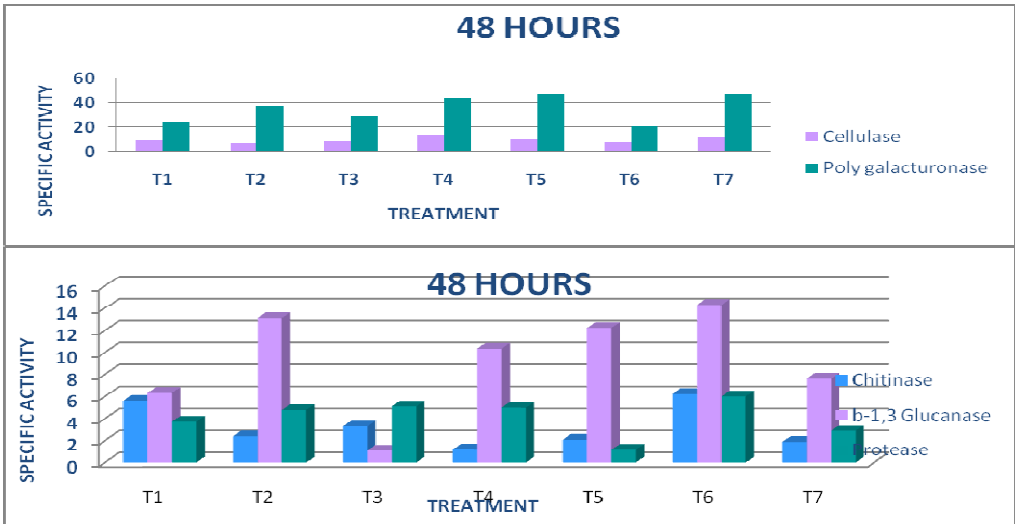

Figure 1. *In-vitro* production of cell wall degrading enzymes (A) and pathogenesis related enzymes (B) in the culture medium at 48 h after inoculation (DAI). T1 = *T. harzianum* NBAII Th 1 *x S. rolfsii;* T2 = *T. hamatum* NBAII Tha-1 *x S.rolfsii;* T3 = *T. koningii* 796 x *S. rolfsii;* T4= *T. pseudo koningii* 2048 x *S. rolfsii*; T5 = *T. virens* NBAII Tvs 12 x *S. rolfsii ;* T6 = *T. viride* NBAII Tv 23 x *S. rolfsii ;* T7 = *S. rolfsii.*

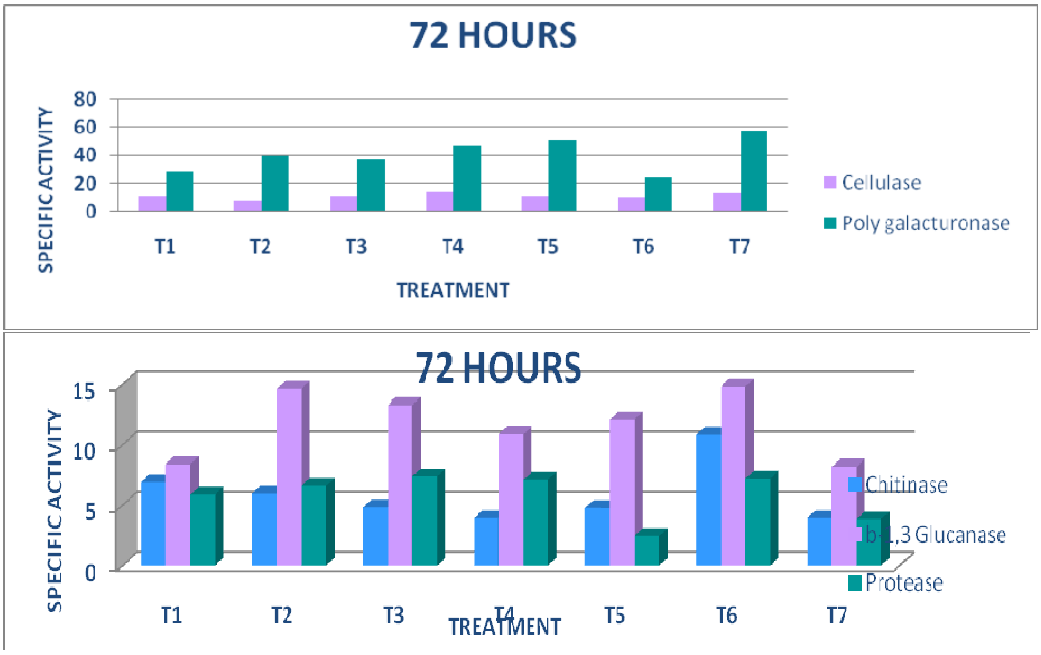

Figure 2. *In vitro* production of cell wall degrading enzymes (A) and pathogenesis related enzymes (B) in the culture medium at 72 h after inoculation (DAI). T1 = *T. harzianum* NBAII Th 1 *x S. rolfsii; T2 = T. hamatum* NBAII Tha-1 *x S. rolfsii; T3 = T. koningii* 796 *x S. rolfsii; T4= T. pseudo koningii* 2048 *x S. rolfsii; T5 = T. virens* NBAII Tvs 12 *x S. rolfsii; T6 = T. viride* NBAII Tv 23 *x S. rolfsii; T7 = S. rolfsii.*

NABII Tv 23(8.34 U.mg^{-1} protein). Protease activity during different time intervals (mean H) showed rise in activity during 48 to 96 h incubation. Combined effect of T×H indicated that the specific activity of protease was higher in *T. viride* NBAII Tv 23. The highest specific activity was recorded in *T. viride* NBAII Tv 23 at all incubation which exceptionally did not inhibit the growth of pathogen effectively. The enzyme activity was more or less correlated

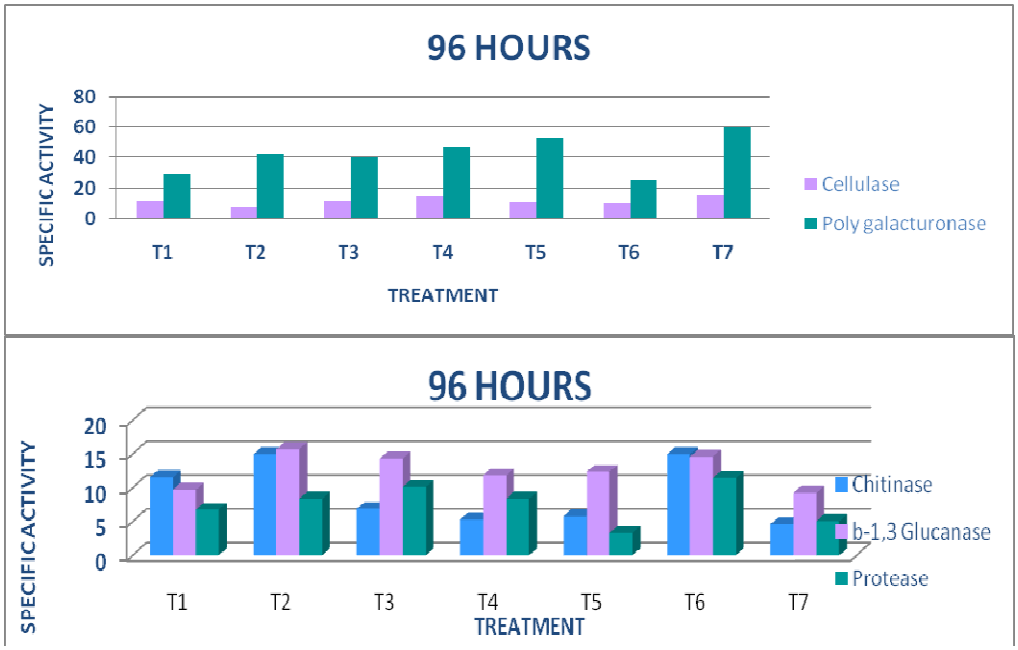

Figure 3. *In vitro* production of cell wall degrading enzymes (A) and pathogenesis related enzymes (B) in the culture medium at 96 h after inoculation (DAI). T1 = *T. harzianum* NBAII Th 1 *xS. rolfsii;* T2 = *T. hamatum* NBAII Tha-1 *x S. rolfsii;* T3 = *T. koningii* 796 *x S. rolfsii;* T4= *T. pseudo koningii* 2048 *x S. rolfsii,* T5 = *T. virens* NBAII Tvs 12 *xS. rolfsii;* T6 = *T. viride* NBAII Tv 23 *x S. rolfsii ;* T7= *S. rolfsii*

Table 2. Specific activity of lytic enzymes β 1,3- glucanase during *in vitro* antagonism of *S. rolfsii* with *Trichoderma* spp. to 4th day after inoculation.

Treatment	β 1, 3-Glucanase (U.mg1protein)			
	48 h	**72 h**	**96 h**	**Mean (T)**
T. harzianum NBAII Th 1 x *S. rolfsii*	6.33	8.43	9.72	8.16
T. hamatum NABII Tha-1 x *S. rolfsii*	13.1	14.64	15.7	14.30
T. koningii MTCC-796 x *S. rolfsii*	1.12	13.34	14.32	12.92
T pseudokoningii MTCC-2048 x *S. rolfsii*	10.34	10.92	11.8	11.02
T. virens NABII TvS 12 x *S. rolfsii*	12.24	12.14	12.36	12.24
T.viride NABII Tv23 x *S. rolfsii*	14.34	14.83	14.59	14.58
S. rolfsii	7.62	8.21	9.25	8.35
Mean (H)	10.72	11.78	12.45	
	S.EM	**C.D at 5%**	**C.V%**	
T	0.061	0.045	0.413	
H	0.010	0.030		
TxH	0.027	0.079		

with growth inhibition of test fungus except *T. viride* NABII Tv 23 and *T. pseudokoningii* MTCC 2048 antagonists.

Considering mean antagonist (T) data, specific activity of cellulase was significantly higher in test fungi control (57.52 U.mg-1 protein) followed by *T. virens* antagonist (Table 6). The significantly minimum cellulase activity (23.60 U.mg-1 protein) was found in culture medium of *T. viride* NBAII Tv 23.

Considering mean antagonist (T) data, specific activity of poly galacturnase was significantly higher in *T. virens* (59.52 $U.mg^{-1}$ protein) followed by *S. rolfsii* (Table 7). The significantly minimum poly galacturnase activity (27.23 $U.mg^{-1}$ proteins) was found in culture medium of *T. viride* NBAII Tv 23.

There are several mechanisms involved in *Trichoderma* antagonism namely antibiosis whereby the antagonistic

Table 3. Specific activity of lytic enzymes chitinase during *in vitro* antagonism of *S. rolfsii* with *Trichoderma* up to 4th day after inoculation (DAI).

Treatment	Chitinase (U.mg-1protein)			
	48 h	**72 h**	**96 h**	**Mean (T)**
T. harzianum NBAIITh 1 X *S .rolfsii*	5.62	6.96	11.49	8.02
T. hamatum NABII Tha-1 X *S .rolfsii*	2.38	5.96	14.94	7.76
T. koningii MTCC-796 X *S. rolfsii*	3.30	4.82	6.84	5.00
T pseudokoningii MTCC-2048 X *S. rolfsii*	1.22	3.91	5.29	3.97
T. virens NABII TvS 12 X *S.rolfsii*	2.08	4.76	15.75	7.53
T.viride NABII Tv23 X *S.rolfsii*	6.23	10.87	14.94	10.68
S. rolfsii	1.89	3.91	4.60	3.46
Mean (H)	3.24	5.88	10.55	
	S.EM	**C.D at 5%**	**C.V%**	
T	0.022	0.065	0.958	
H	0.015	0.042		
TxH	0.039	0.113		

Table 4. Specific activity of lytic enzymes protease during *in vitro* antagonism of *S. rolfsii* with *Trichoderma* up to 4th day after inoculation (DAI).

Treatment	Protease (U.mg-1protein)			
	48 h	**72 h**	**96 h**	**Mean (T)**
T. harzianum NBAII Th 1 x *S. rolfsii*	3.73	5.91	6.73	5.45
T. hamatum NABII Tha-1x *S. rolfsii*	4.77	6.63	8.42	6.60
T. koningii MTCC-796 x *S. rolfsii*	5.08	7.46	10.1	7.54
T pseudokoningii MTCC-2048 x *S. rolfsii*	4.97	7.15	8.42	6.84
T. virens NABII TvS 12 x *S. rolfsii*	1.2	2.49	3.37	2.36
T.viride NABII Tv23 x *S. rolfsii*	6.01	7.25	11.38	8.34
S. rolfsii	2.87	3.83	5.05	3.65
Mean(H)	3.98	5.81	7.69	
	S.EM	**C.D at 5%**	**C.V%**	
T	0.018	0.053	0.966	
H	0.012	0.035		
TxH	0.032	0.093		

fungus shows production of antibiotics, competition for nutrients. In the case of mycoparasitism, *Trichoderma* directly attacks the plant pathogen by excreting lytic enzymes such as chitinases, β-1,3 glucanases and proteases (Haran et al., 1996). Due to the fact that the skeleton of pathogenic fungi cell walls contains chitin, glucan and proteins, enzymes that hydrolyze these components have to be present in a successful antagonist in order to play a significant role in cell wall lysis of the pathogen (Lorito et al., 1994; Carsolio et al., 1999). Filamentous fungal cell wall also contains lipids and proteins (Hunsley and Burnett, 1970). It therefore, was expected that antagonistic fungi synthesized proteases which may act
on the host cell-wall (Howell, 2003).

Most of the bio-control agents are known to produce chitinase and β-1,3-glucanases enzymes which could degrade the cell wall leading to the lysis of hyphae of the pathogen (Sangle and Bambawale, 2004). The pathogen cell-wall and chitin induce *nag1* gene, but it is only triggered when there is contact with the pathogen (Howell, 2003; Harman et al., 2004; Lorito et al., 1994). Present findings showed higher specific activity of enzymes-chitinase and β-1, 3 glucanase in *Trichoderma* spp. incubated with pathogen cell wall. Activity of these enzymes varied with various *Trichoderma* species. This may be due to the expression of certain gene in *Trichoderma* spp. during incubation as *Chit33* is expressed only during the contact phase and not before overgrowing *Rhizoctonia solani* (Dana et al., 2001).

However, *chit36Y* does not need the direct contact of the pathogen to be expressed. *Chit33*, *chit42* and *chit36*

Table 5. Specific activity of lytic enzymes poly galacturonase during *in vitro* antagonism of *S. rolfsii* with *Trichoderma* up to 4th DAI.

Treatment	Poly galacturonase(U.mg-1protein)			
	48 h	**72 h**	**96 h**	**Mean (T)**
T. harzianum NBAII Th 1 x *S. rolfsii*	24.48	28.59	29.17	27.41
T. hamatum NABII Tha-1 x *S. rolfsii*	37.38	39.29	42.08	39.58
T. koningii MTCC-796 x *S.rolfsii*	29.03	37.24	40.17	35.48
T. pseudokoningii MTCC-2048 x *S. rolfsii*	43.98	46.77	47.06	45.93
T. virens NABII TvS 12 x *S. rolfsii*	47.35	50.87	53.36	59.52
T. viride NABII Tv23 x *S. rolfsii*	21.26	24.04	25.51	23.60
S. rolfsii	47.5	57.76	60.4	55.22
Mean (H) :-	35.85	40.65	42.53	
	S.EM	**C.D at 5%**	**C.V%**	
T	0.074	0.074	0.561	
H	0.048	0.048		
TxH	0.128	0.360		

Table 6. Specific activity of lytic enzymes cellulase during *in vitro* antagonism of *S. rolfsii* with *Trichoderma* up to 4th DAI.

Treatment	Cellulase (U.mg-1protein)			
	48 h	**72 h**	**96 h**	**Mean (T)**
T. harzianum NBAII Th 1x *S. rolfsii*	223.9939	23.38	24.17	26.25
T. hamatum NABII Tha-1 x *S. rolfsii*	36.38	39.29	40.08	38.58
T. koningii MTCC-796 x *S. rolfsii*	29.33	37.24	42.17	36.24
T pseudokoningii MTCC-2048 x *S.rolfsii*	39.98	44.77	48.06	44.30
T. virens NABII TvS 12 x *S. rolfsii*	45.35	50.87	54.36	49.52
T.viride NABII Tv23 x *S. rolfsii*	20.26	23.04	25.51	22.93
S. rolfsii	46.6	60.76	66.4	57.92
Mean(H)	35.85	40.65	42.53	
	S.EM	**C.D at 5%**	**C.V%**	
T	0.074	0.074	0.561	
H	0.048	0.048		
TxH	0.128	0.360		

have been over expressed in *Trichoderma* spp. in order to test the role of these chitinases in mycoparasitism, and the 42-kDa chitinase is believed to be a key enzyme (Howell, 2003). The production of β-1, 3 glucanase was also reported as an important enzymatic activity in bio-control of pathogen because β-1, 3 glucan is a structural component of fungal cell walls. Many β-1,3-glucanases have been isolated, but only a few genes have been cloned, for example, bgn13.1 (Collmer, 1988).

Correlation between antagonism study and release of cell wall degrading enzymes by *Trichoderma* spp. using pathogen cell wall as substrate

A highly significant positive correlation (p=0.01) was observed between percent growth inhibition of pathogen *S. rolfsii* and chitinase and β-1, 3-glucanase enzymes (Table 7) which established a relationship to inhibit the growth of fungal pathogen by increasing the levels of these cell wall degrading enzymes during antagonism. Protease showed positive but no significant correlation among percent growth inhibition, chitinase and β-1,3-glucanase enzymes. The significant negative correlation (p=0.05) was established between growth inhibitions of test fungus and cellulase, suggesting that percent growth inhibition of pathogen decreased with increasing concentration of cellulase during antagonism. Cellulase was significantly negatively correlated with pathogenesis related enzymes mainly chitinase and β-1,3-glucanase. Among chitinase and β-1,3-glucanase, the correlation was highly positive, indicating that these enzymes worked together in synergistic cooperation to inhibit the growth of pathogen.

Table 7. Correlation matrix between percent growth inhibition of *S. rolfsii* and production of lytic enzymes during antagonism in the culture medium at 5th DAI.

Lytic enzymes	Inhibition	Cellulase	Poly-galacto	Chitinase	b-1,3- Glucanase	Protease
Inhibition	1.000					
Cellulase	-0.674	1.000				
Poly-galacto	-0.777	0.536**	1.000			
Chitinase	0.587**	-0.827	-0.777	1.000		
b-1,3 Glucan	0.590**	-0.753	-0.362	0.561**	1.000	
Protease	0.475*	-0.313	-0.700	0.545**	0.620**	1.000

Therefore, it can be summarized that among seven *Trichoderma* spp., *T. viride* NBAII Tv 23 was the best agent to inhibit the growth of the pathogen *S. rolfsii* during antagonism study on PDA media. Percent growth inhibition of pathogen and production of cell wall degrading enzymes-chitinase and β-1,3 glucanase correlated positively, suggesting that these enzymes were released during antagonism and inhibited the growth of fungal pathogen *S. rolfsii*. Thus, *T. viride* NBAII Tv 23 is the most suitable strain to be used in biological control of plant pathogen *S. rolfsii*, causing stem rot disease in groundnut.

Conflict of interest

The authors did not declare any conflict of interest.

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