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In vitro antagonistic activity of *Trichoderma* isolates against *Rhizoctonia solani* by production of lytic enzymes

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Abstract

Twenty-six *Trichoderma* isolates were tested for their potentiality in the production of cell wall degrading enzymes and mycoparasitic activity against *Rhizoctonia solani*, sheath blight of rice. Statistically significant variation was observed in production of lytic enzymes by *Trichoderma*. In the production of cellulase, eleven *Trichoderma* isolates showed positive result. Among these, the maximum cellulase production was observed with *T. reesei* (TR), followed by *T. longibrachiatum* (M6Ms). Thirteen *Trichoderma* isolates were positive in amylase enzyme production. In the assay *T. longibrachiatum* (M9Ms) produced maximum amylase, followed by *T. longibrachiatum* (M3Ps). In the chitinase assay highest producer was *T. longibrachiatum* (M1Ps), followed by *T. asperellum* (M4Ps). Eight isolates of *Trichoderma* produced protease enzyme viz., *T. asperellum* (M4Ps), *T. aureoviride* (TA1),

T. reesei (TR), *T. longibrachiatum* (TL3, M1Ps, M5Ms, M8Ps), *T. virens* (TV1). Among this, maximum protease producer was *T. aureoviride* (TA1) and the least producer was *T. longibrachiatum* (M5Ms). When the total enzyme activity of cellulase and amylases was compared it clearly showed that only seven isolates of *Trichoderma* produced both enzymes.

T. asperellum (M3M) and *T. reesei* (TR) produced all the three enzymes, whereas *T. aureoviride* (TA3), *T. longibrachiatum* (TL1, M3P), *T. virens* (TV1) and *T. viride* (TD1) did not show any enzyme activity. Four isolates of *Trichoderma*, belonging to *T. harzianum* (M10P), *T. koningii* (TR), *T. longibrachiatum* (M6Ms) and *T. viride* (M1M) showed maximum mycoparasitism on *R. solani* than other 22 isolates which showed moderate and minimum mycoparasitic activity. A positive correlation was observed between mycoparasitic activity against *R. solani* and production of cell wall degrading enzymes (CWDEs) by *Trichoderma*. *T. reesei* (TR) and *T. longibrachiatum* (M1Ps) was found to be the best strains to be used as a bio control agent to control rice pathogen *R. solani*

Keywords: *Trichoderma*, *Rhizoctonia solani*, mycoparasitism, lytic enzymes.

Introduction

Sheath blight is considered to be an important disease of rice next to rice blast (DRR 2007). It is caused by a soil borne fungus *Rhizoctonia solani* (Teleomorph *Thanetophorus cucumeris* (A B Frank, Donk, Ou 1985) [37]. The loss of yield due to this disease has been estimated to be 10-50% in India. The disease appears both in the nursery and on transplanted rice (Banniza and Holderness 2001) [3], inflicting heavy losses in many Asian countries and it is most serious fungal diseases worldwide (Manalo *et al.* 2001) [31]. The sclerotia of *R. solani* remain viable in the soil for several years and it causes spots or lesions mostly on the leaf sheath (Ou 1985; Damicone *et al.* 1993) [10]. Its wide occurrence is brought about by intensive methods of rice cultivation involving the use of short culmed, high tillering, high yielding varieties, high inputs of nitrogen fertilizer and application of fungicides. Nevertheless, fungicides have many undesirable attributes, novel bio control agents have therefore been looked for and *Trichoderma* species have been the preferred choice. Several modes of action have been proposed to explain the bio control of plant pathogens by *Trichoderma* these include production of antibiotics and cell wall degrading enzymes, mycoparasitism, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of these possibilities (Kubicek *et al.* 2019) [29]. *Trichoderma* spp.

are considered to inhibit the growth of phytopathogenic fungi via the production of hydrolytic enzymes which degrade the cell walls of the pathogens (Yao *et al.* 2023) ^[48]. Mycoparasitic activity is one of the major mechanisms proposed to explain the antagonistic activity of *Trichoderma* spp. against many soil borne plant pathogenic fungi (Weindling 1932, 1934; Elad *et al.* 1982, Lynch 1990, Melo and Faull 2000). This has been long considered as an important means of action of bio control by *Trichoderma* spp., and is a complex process that involves growth of the biocontrol agent towards the target fungi, lectin mediated coiling of attachment of *Trichoderma* hyphae to the pathogen, and finally attack the target fungus by the dissolution of cell wall of the pathogen by the activity of enzymes. The active principle responsible for mycoparasitic activity is by the production of either toxins or hydrolytic enzymes or both by *Trichoderma* (Howell 2003) ^[23]. The parameter for the successful use of *Trichoderma* as a potential biocontrol agent is its efficient coiling process followed by a substantial production of hydrolytic enzymes. Synergism between hydrolytic enzymes and antibiotics that affect cell wall synthesis facilitates the penetration of further active compounds suppressing fungal growth (Witkowska and Maj 2002) ^[45]. *Trichoderma* spp. was shown to be very efficient producers of extracellular enzymes, and some of these have been implicated in the biological control of plant diseases (Monte, 2001 ^[35]; Harman, 2006 ^[21]; Jayalakshmi *et al.* 2009) ^[24]. The cell wall degrading enzymes such as chitinases, cellulases, β -1,3-glucanases, and proteases have been associated with the ability of *Trichoderma* spp. to control plant pathogens (De Marco *et al.* 2003) ^[13]. As the growth control of the pathogen is mostly by several strains of *Trichoderma*. However, the quantitative production of these enzymes has an effect directly on lysis of the cell walls of the pathogens. So, the products of lytic enzymes to be identified, to select as an efficient isolate for field application. Hence, a study was undertaken to evaluate the potential of *Trichoderma* isolates to produce hydrolytic enzymes.

Materials and Methods

Mycoparasitic activity of *Trichoderma* isolates against *R. solani*

This study was conducted using dual culture method (Dennis and Webster 1971c) with modifications. The 5 mm mycelial disc of *Rhizoctonia solani* was placed on 2% water agar medium and after one day of incubation a 5 mm disc of *Trichoderma* inoculum was placed on the inoculum of test pathogen. A block of medium (10 × 20 cm) was cut at the juncture of two colonies and mycelium of the two fungi was mounted in lactophenol, and examined under microscope for hyphal interactions.

Preliminary screening test for production cellulase by *Trichoderma* isolates

The preliminary screening for cellulase enzyme production by *Trichoderma* was assayed in Czapek-Mineral salt agar medium and the pH was adjusted to 6.5. *Trichoderma* isolates were inoculated on the Petri plates and incubated at room temperature (27±2°C) for 2-5 days. Then, the plates were flooded with 1% aqueous solution of hexadecyltrimethyl ammonium bromide. The plates were observed for the formation of a clear zone around the fungal growth (Aneja 1996) ^[2].

Cellulase assay: To study the cellulase production of the *Trichoderma* isolates, Czapek-Dox broth medium with 10g/l Carboxy Methyl Cellulose (1% CMC) was employed. Ten drops of Tween-80 was added. The pH of the medium was adjusted to 5.5, dispensed into 100 ml conical flasks and autoclaved. Later, one ml (2 X10⁷/ ml) spore suspension of each *Trichoderma* isolate were inoculated and incubated in orbital shaker (120 rpm) at room temperature (27±2°C) for seven days.

Harvest the culture filtrate and the supernatant was used as enzyme source. CMC activity was determined by 1 ml of enzyme solution with 10 mg of CMC diluted with acetate buffer, pH 6.5, and incubated for 30 min at 60°C. To this enzyme solution, DNS reagent was added and the optical density was measured at 520 nm with a UV-visible spectrophotometer (UV 1100, Shimadzu). The reducing sugars were estimated by DNS method as described by Miller (1959) ^[33].

Preliminary screening test for production amylase by *Trichoderma* isolates:

Starch Agar medium was used for the preliminary screening of amylase and the pH was adjusted to 7.0. The Petri plates were inoculated with the *Trichoderma* species and incubated at room temperature (27±2°C) for 2-5 days. Then, the plates were flooded with Grams iodine solution and the plates were observed for the formation of a clear zone around the fungal growth (Aneja 1996) ^[2].

Amylase assay: To study the amylase activity of the *Trichoderma* isolates, Czapek-Dox broth medium with 10g/l soluble starch (1% soluble starch) was employed. Ten drops of Tween-80 was added. The pH of the Czapek-Dox broth medium was adjusted to 5.5, dispensed into 100 ml conical flasks and autoclaved. Later, one ml (2 X10⁷/ ml) spore suspension of each *Trichoderma* isolate were inoculated and incubated in orbital shaker (120 rpm) at room temperature (27±2°C) for seven days. Harvest the culture filtrate and the supernatant was used as enzyme source. Amylase activity was determined by 1 ml of enzyme solution with 1 ml of 1% soluble starch in acetate buffer, pH 6.5, and incubated for 30 min at 37°C. After incubation period add NaOH and reducing sugars were estimated by DNS method as described by Miller (1959) ^[33].

Preliminary screening for chitinases: *Trichoderma* isolates were grown on synthetic medium where carbon source was substituted by colloidal chitin and incubated at room temperature. Controls were maintained with and without any carbon source. Colloidal chitin for enzymatic studies was prepared according to the procedure of Berger and Reynolds (1958) ^[5].

Chitinase assay: Chitinase- β -1,4-Poly N-Acetyl D-glucosaminidase assay was carried out by Reissing *et al.* (1959) ^[38]. The 200 μ l reaction mixture containing 1% chitin, 20 μ g of protein (crude enzyme) in 0.1M acetate buffer (pH 5.0) is incubated for the desired time in water bath shaker (100 rpm) at 37°C. The reaction is stopped by boiling at 100°C and then centrifuged at 10,000 rpm for 10 min; the supernatant is used for assay. OD values were taken at 580 nm. Specific activity of chitinase was expressed as μ moles of Glc NAc released/ mg of protein/ h.

Protease assay: Protease assay was done by following the modified procedure of Anson's method (Yang and Huang 1994) [47]. The substrate used (1% casein in 50mM phosphate buffer, pH 7.0) was denatured at 100°C for 15min in a water bath and cooled at room temperature. The reaction mixture containing 1ml of substrate and 1ml of enzyme solution were incubated at 37°C for 20 min with shaking and was stopped with 3ml of 10% trichloroacetic acid (TCA). The absorbance of the liberated tyrosine in the filtrate was measured at 280nm. One unit of protease activity was defined as the amount of enzyme that produced an absorbance at 280nm equivalent to 1μmol of tyrosine in one minute under the assay condition. Average value of triplicate data was presented.

Results

Twenty-six isolates of *Trichoderma* were obtained from the rice soil samples collected from two districts (Nalgonda and Ranga Reddy) of Telangana and forest soil samples of Uttaraanchal (Chamoli), India. A preliminary qualitative assay was conducted to screen 26 *Trichoderma* isolates for mycoparasitic activity against *R. solani* and cell wall degrading enzymes production (CWDEs) i.e., Cellulases, Amylases, Chitinases and Proteases. Based on the results, the isolates were subjected to quantify the enzymes in *in*

vitro. All the twenty-six isolates of *Trichoderma* showed some degree of mycoparasitic activity against the test pathogen *Rhizoctonia solani*. The degree of hyphal coiling is varied with different isolates (Table 1).

T. harzianum (M10P), *T. koningii* (TR), *T. longibrachiatum* (M6Ms) and *T. viride* (M1M) showed maximum mycoparasitism (+++) on *R. solani* (Figure 1), the intense coiling of *Trichoderma* hyphae around the mycelium of the pathogen was observed which led to the distortion of pathogen hyphae, finally the death of the *R. solani* mycelium was noticed.

T. asperellum (TS, M3M, M4Ps), *T. aureoviride* (TA4), *T. harzianum* (TH1, TH2), *T. longibrachiatum* (TL1, TL2, TL3, M1Ps, M3P, M3Ps, M5Ms, M8Ps) and *T. viride* (TD1, TD2) showed moderate mycoparasitic activity with less hyphal coiling, moderate mycoparasitism (++), mycelial growth of *Trichoderma* along with the pathogen hyphae was observed, but the intensity of coiling is less and distortion of the pathogen hyphae is moderate. In minimum mycoparasitic activity (+), the hyphal coiling of *Trichoderma* around the pathogen and distortion of the pathogen was very less. The isolates are *T. aureoviride* (TA2, TA3), *T. longibrachiatum* (M9Ms) and *T. virens* (TV1, TV2) and the occurrence of parallel mycelial growth of *Trichoderma* isolates against *R. solani* hyphae was rare in the test plate (Figure 1).

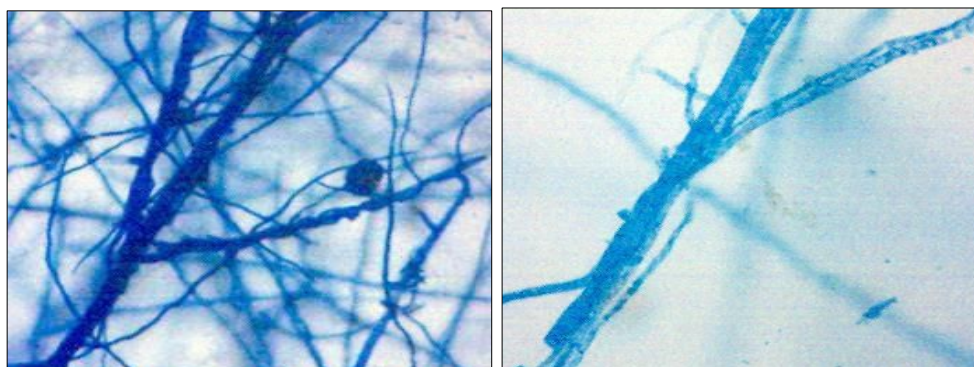


Fig 1: Mycoparasitism of *Trichoderma* spp.

A. *T. koningii* (TR) showing coiling on *Rhizoctonia solani* hyphae

B. *T. virens* (TV1) showing parallel growth on the *Rhizoctonia solani* hyphae. Eleven out of twenty-six *Trichoderma* isolates showed positive result in the production of cellulase. The strains positive for the production test were *T. asperellum* (TS, M3M), *T. aureoviride* (TA1, TA4), *T. harzianum* (TH2, M10P), *T. reesei* (TR), *T. longibrachiatum* (M6Ms), *T. virens* (TV2), and *T. viride* (TD2, M1M). These are further analyzed by quantitative enzyme assay (Table 1). Among these isolates, the maximum cellulase production was observed with *T. reesei* (TR) (0.433 U/mL/1'), followed by *T. longibrachiatum* (M6Ms) (0.397 U/mL/1'). The least activity was recorded with *T. virens* (TV2) (0.029 U/mL/1'). Thirteen isolates of *Trichoderma* spp. were positive in amylase enzyme production and these belong to *T. asperellum* (TS, M3M), *T. aureoviride* (TA4), *T. harzianum* (TH1, TH2), *T. reesei* (TR), *T. longibrachiatum* (TL2, TL3, M1Ps, M3Ps, M9Ms), *T. virens* (TV2), and *T. viride* (TD2). In the assay *T. longibrachiatum* (M9Ms) produced maximum amylase, followed by *T. longibrachiatum* (M3Ps) (Table 1). In the preliminary screening test, twelve isolates

of *Trichoderma* showed chitin degrading ability. Among these six isolates *T. asperellum* (M3M, M4Ps), *T. aureoviride* (TA2), *T. reesei* (TR), and *T. longibrachiatum* (M1Ps, M8Ps) completely degraded the chitin (Fig 2).

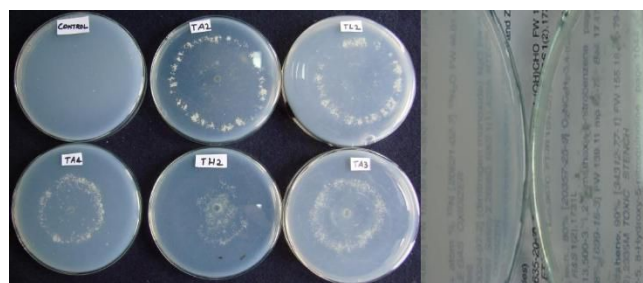


Fig 2: *Trichoderma* isolates showing chitin degradation in chitin amended medium

Right: *T. reesei* test plate is transparent because of chitin degradation

Left: Control plate is opaque because of no chitin degradation. Chitinase assay was done for these six isolates of *Trichoderma*. The highest of chitinase produced by *T. longibrachiatum* (M1Ps) (521.6 Glc Nac mg protein/h'),

followed by *T. asperellum* (M4Ps) (386.7 Glc Nac mg protein/h'). Eight isolates of *Trichoderma* produced protease enzyme viz., *T. asperellum* (M4Ps), *T. aureoviride* (TA1), *T. reesei* (TR), *T. longibrachiatum* (TL3, M1Ps, M5Ms M8Ps), *T. virens* (TV1). Maximum protease production was observed with *T. aureoviride* (TA1) (590µg/ml) and the least producer was *T. longibrachiatum* (M5Ms) (360µg/ml)

(Table 1). *T. asperellum* (M3M) and *T. koningii* (TR) produced all the three enzymes, whereas *T. aureoviride* (TA3), *T. longibrachiatum* (TL1, M3P), *T. virens* (TV1) and *T. viride* (TD1) did not show any enzyme activity. A positive correlation was found between mycoparasitic activity and Cell Wall Degrading Enzymes production (CWDEs).

Table 1: Cell wall Degrading Enzymes (CWEDs) production by *Trichoderma* isolates

<i>Trichoderma</i> species	Isolate No	Amylase activity (U/mL/1')	Cellulase activity (U/mL/1')	Chitinase activity (Glc Nac mg protein/h')	Protease activity (µg/ml)	Mycoparasitic activity
<i>T. asperellum</i>	TS	0.030	0.062	0	0	++
<i>T. asperellum</i>	M3M	0.068	0.166	326.1	0	++
<i>T. asperellum</i>	M4Ps	0	0	386.7	570	++
<i>T. aureoviride</i>	TA1	0	0.083	0	590	++
<i>T. aureoviride</i>	TA2	0	0	173.5	0	+
<i>T. aureoviride</i>	TA3	0	0	0	0	+
<i>T. aureoviride</i>	TA4	0.017	0.056	0	0	++
<i>T. harzianum</i>	TH1	0.017	0	0	0	++
<i>T. harzianum</i>	TH2	0.025	0.035	0	0	++
<i>T. harzianum</i>	M10P	0	0.150	0	0	+++
<i>T. reesei</i>	TR	0.169	0.433	368.9	490	+++
<i>T. longibrachiatum</i>	TL1	0	0	0	0	++
<i>T. longibrachiatum</i>	TL2	0.026	0	0	0	++
<i>T. longibrachiatum</i>	TL3	0.029	0	0	470	++
<i>T. longibrachiatum</i>	M1Ps	0.112	0	521.6	470	++
<i>T. longibrachiatum</i>	M3P	0	0	0	0	++
<i>T. longibrachiatum</i>	M3Ps	0.176	0	0	0	++
<i>T. longibrachiatum</i>	M5Ms	0	0	0	360	++
<i>T. longibrachiatum</i>	M6Ms	0	0.397	0	0	+++
<i>T. longibrachiatum</i>	M8Ps	0	0	359.5	470	++
<i>T. longibrachiatum</i>	M9Ms	0.221	0	0	0	+
<i>T. virens</i>	TV1	0	0	0	450	+
<i>T. virens</i>	TV2	0.007	0.029	0	0	+
<i>T. viride</i>	TD1	0	0	0	0	++
<i>T. viride</i>	TD2	0.034	0.117	0	0	++
<i>T. viride</i>	M1M	0	0.071	0	0	+++
CD ($p \leq 0.05$)		0.002	0.0023	1.50		
CV (%)		2.57	2.33	1.11		

Discussion

Trichoderma spp. produces several hydrolytic enzymes such as cellulase, β -1,3-glucanase, amylase, protease and chitinase which play an important role in the antagonistic activity on plant pathogens through mycoparasitism, degradation of cell wall components and lysis of the host (Markovich and Kononova 2003; Cherif and Benhamou 1990).

Mycoparasitism of various *Trichoderma* isolates on *R. solani* showed variation in degree of coiling or parallel growth. *T. harzianum* (M10P), *T. koningii* (TK) and *T. viride* (M1M) recorded the highest mycoparasitic activity (Table.1). The studies on mycoparasitism by fifteen *T. harzianum* strains against *R. solani* exhibited that coiling around the hyphae of *R. solani* followed by substantial production of hydrolytic enzymes (Almeida 2007). Risoli and co-workers (2022) ^[39] reported that *Trichoderma* is capable of producing putrescence under specific environmental conditions, which contributes to its mycoparasitic activity against *Botrytis cinerea*. Chet and Baker (1981) suggested the involvement of cellulase production in mycoparasitism on *Pythium* spp. by *T. hamatum*. The isolates belonging to *T. aureoviride* (TA1, TA2), *T. longibrachiatum* (M9Ms) and *T. virens* (TV1) are non-producers of cellulase (Table.1) and *T. virens* (TV2) showed slight production. This may be the reason for least

mycoparasitic activity which did not allow the antagonist to dissolve cell walls of *R. solani*. This study showed correlation between cellulase production and mycoparasitic activity. Melo *et al.* (1997) reported high levels of cellulases that hydrolyse the cell walls of other fungi. The test pathogen *R. solani* cell wall consists of high levels of glucans.

Hence, these antagonistic *Trichoderma* isolates are capable of hydrolyzing the cell walls of the pathogen by the production of lytic enzymes (cellulase) which might have led to the coiling and penetration of the test antagonist. The high mycoparasitic activity with the four isolates of *Trichoderma* (M10P, TK, M6Ms, M1M) was at later stages resulted in the hyphal collapse of *R. solani*. In 1983 Elad *et al.* proved the localized cell wall lysis on the point of interaction between *Trichoderma* species and *R. solani* by scanning electron microscopy and fluorescent microscopy studies.

Barbosa *et al.* (2001) ^[4] reported that *T. reesei* is a high producer of cellulase when compared with other *Trichoderma* isolates and *T. harzianum*, *T. reesei*, *T. polysporum*, and *T. viride* isolates produced amylases and it is one of the enzymes partially necessary for the growth of *Trichoderma*. In this study also *T. reesei* (TR) was the highest producer of cellulase. Krishnamurthy *et al.* (1990) ^[28] screened 35 strains of *T. viride* and *T. harzianum* for

antagonism against *R. solani* and reported that four strains were better for chitinase induction with colloidal chitin. In this study 6 out of 26 isolates showed chitinase activity belonging to the species *T. reesei* (TR), *T. Longibrachiatum*, *T. asperellum* and *T. aureoviride*. Ulhoa and Peberdy (1991)^[43] selected *T. harzianum* as an effective chitinase producer. Purified chitinolytic enzymes of *T. harzianum*, inhibited a wide range of fungi (Harman *et al.* 1993^[20]; Lorito *et al.* 1993)^[30].

The isolates *T. reesei* (TR) and *T. longibrachiatum* (M1Ps) inhibited the growth of *R. solani* and completely inhibited the sclerotial formation in dual culture than any other selected isolates (Chakravarthy and Nagamani 2007)^[9]. *T. asperellum* (M4Ps), *T. longibrachiatum* (TL3, M9Ms), *T. viride* (TD2) isolates inhibited growth of *R. solani* through the production of volatile antibiotics, whereas *T. reesei* (TR), *T. longibrachiatum* (M1Ps, M8Ps) showed least inhibition. But among the three isolates only *T. reesei* (TR) and *T. longibrachiatum* (M1Ps) inhibited sclerotial formation completely through the production of volatile metabolites (Chakravarthy and Nagamani 2007)^[9]. Several studies have demonstrated that certain *Trichoderma* species are capable of producing volatile metabolites that can inhibit colony growth to varying extents, with some strains achieving inhibition rates exceeding 80% (Thambugala *et al.* 2020^[42]; Kong *et al.* 2022)^[26].

All the three enzymatic activities were observed with the isolate *T. reesei* (TR). *T. longibrachiatum* (TL3 and M9Ms) showed only amylase activity, whereas by *T. asperellum* (M4Ps) and *T. longibrachiatum* (M8Ps) isolates only chitinases enzyme activity was recorded. With M1Ps enzyme activity of both cellulase and chitinase was observed, but with TD2 isolate cellulase and amylases activity was noticed. *T. longibrachiatum* (M1Ps) recorded highest chitinase enzyme activity and *T. reesei* (TR) is the highest cellulase enzyme producer when compared to other isolates.

Moreover, Elad *et al.* (1982) reported that the isolates of *T. harzianum*, which were found to differ in their ability to attack *S. rolfii*, *R. solani* and *P. aphanidermatum*, also differed in the levels of mycolytic enzymes produced by them. The enzymes chitinases, α -1, 3, β -1, 3 and β -1, 6 glucanases, and proteases, that degrade fungal cell walls (Ait-Lahsen *et al.* 2001^[1]; De la Cruz *et al.* 1995a)^[11, 12]. Ghasemi *et al.* 2020^[18] reported that there was a strong link between the diversity of various chitinase proteins and the antagonistic properties of the *T. virens* mutant M8 against *R. solani*.

Fungal proteases may also be significantly involved in cell wall degradation, since fungal cell walls contain chitin and glucan polymers embedded in and covalently linked to a protein matrix (Kaptein *et al.* 1996)^[25]. These observations, together with the fact that chitin and glucans are the main polysaccharides of fungal mycelia, suggest that chitinases and glucanases play an essential role in the lysis of phytopathogenic fungal cell walls during antagonism (Ait-Lahsen *et al.* 2001^[1]; Bosah *et al.* 2010^[8]; De la Cruz *et al.* 1995b)^[11, 12]; Henis and Chet 1975)^[22]. The role of

Trichoderma proteases in biological control as well as their advantages and disadvantages in biotechnology has been reviewed (Kredics *et al.* 2005)^[27]. These enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, there by destroying cell wall integrity (Howell 2003)^[23].

Involvement of proteases in bio control processes has already been reported (Elad and Kapat 1999)^[15]. Besides carbohydrate – active enzymes, proteases are also important for degradation of fungal cell wall and it was evident from the experiments with *T. atroviride* and *T. virens* by Gruber and Seidl-Seiboth (2012)^[40]. Different types of proteases were reported to be induced during mycoparasitism before and during contact with the prey in different *Trichoderma* species (Geremia *et al.* 1993^[17]; Seidl *et al.* 2009^[40]; Suarez *et al.* 2007)^[41].

Although some of the other isolates exhibited superior production of volatile and non-volatile compounds, they were not selected for further investigation, as the primary focus of this study was on cell wall degrading enzyme activity and mycoparasitic potential against *Rhizoctonia solani*. Hence, in the present investigation out of twenty six isolates of *Trichoderma*, two strains namely *T. reesei* (TR) and *T. longibrachiatum* (M1Ps) were selected because these two isolates showed complete inhibition of sclerotial formation and produced high quantities of chitinase and non-volatiles.

Conclusion

Twenty six *Trichoderma* isolates screened for their ability to produce cell wall degrading enzymes (CWDEs) and their mycoparasitic activity against *Rhizoctonia solani*, the causative agent of sheath blight in rice. A significant variability was observed among the isolates in the production of enzymes such as cellulase, amylase, chitinase, and protease. Among the isolates, *T. reesei* (TR) and *T. longibrachiatum* (M1Ps) exhibited highest levels of enzyme production and were among the few strains of *Trichoderma* capable of producing all three key enzymes. Furthermore, these strains exhibited strong mycoparasitic activity against *R. solani*, showing a clear positive correlation between CWDE production and biocontrol potential. Considering all aspects of our study, *T. reesei* (TR) and *T. longibrachiatum* (M1Ps) are identified as the most promising biocontrol agents for sheath blight management in rice.

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