The Bioscan 12(1): 129-134, 2017
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF TRICHODERMA SPP. IN DIFFERENT CROPPING SYSTEMS OF CHITTOOR DISTRICT (A.P)

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KEYWORDS
*Trichoderma*spp.
Morphological
Biochemical
Characterization
Chitinase
Glucanase

Received on :
06.11.2016

Accepted on :
09.02.2017

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ABSTRACT
Nine isolates of *Trichoderma* were isolated from different cropping systems (Groundnut, Tomato and Red gram) of Chittoor district (A.P) and characterized based on morphological, biochemical characters. Based on growth rate, the isolates GRT-2, GRT-3, TRT-2, RRT-2 with 90 mm diameter radial growth within 48 h were categorized as very fast growing and GRT-1, RRT-1, TRT-1 which exhibited 90 mm within 72 h as fast growing, whereas the isolates GRT-4 and GRT-5 exhibited 90 mm within 96h as medium growing isolates. Isolates GRT-2, GRT-4, GRT-5 identified as *Trichoderma virens,* RRT-1 and GRT-3 as *Trichoderma harzianum*. TRT-2 and RRT-2 as *Trichoderma asperillum*. Isolates GRT-1 and TRT-1 as *Trichoderma longibrachiatum* and *Trichoderma pseudokoningii* respectively level based on the morphological characters and conidiophores branching, conidiation, conidia shape under microscope. Among nine isolates of *Trichoderma* spp, the isolate GRT-3 recorded highest optical density of 0.290, 0.130, 0.260 in terms of production of chitinase, â-1,3 glucanase and cellulase enzyme activity. When compared to other teste isolates, isolate GRT-3 recorded as very fast growing and also have more enzyme activity. Trichoderma spp. in different cropping systems showed variability in morphologically and biochemically by producing range hydrolytic enzymes. So, they can be used as a fungal antagonists against soil borne pathogens.

INTRODUCTION

Genus *Trichoderma* have traditionally been classified as Fungi Imperfecti based upon differences in morphology under Deuteromycotina, Hyphomycetes, Phialasporace, Hyphales, Dematiaceae as they produce only asexual spores *i*.e. conidia (Singh *et al*., 2006). As is usually the case with other fungal genera, species of *Trichoderma* too were defined originally on the basis of morphology by workers like Rifai (1969) and Bisset (1991a, b and c). The culture sporulation pattern varied considerably within and between the species. Although conidial shape and arrangement and hyphal branching pattern helped in distinguishing species from each other. However, sporulation pattern and size of the spores within the species were highly variable (Sharma and Singh, 2014). There are several antagonistic mechanisms used by *Trichoderma*, mainly antibiosis and mycoparasitism where by biocontrol agent directly attack the pathogen by secreting lytic enzymes such as β-1, 3 glucanase, chitinase and cellulase and protease(Khushwaha and Verma, 2014).As the skeleton of fungal cell wall mainly contains chitin, glucan and proteins. The enzymes that hydrolyse these components are one of the main mechanisms accounting for showing antagonistic activity against plant pathogenic fungi (Lunge and Patil, 2012). *Trichoderma* spp are most commonly use biocontrol agent against various plant diseases specially soil borne pathogens. It offers a chance to improve crop production in sustainable way and avoid the resistance of chemical pesticides to the target pathogens (Devi and Sinha, 2014). Seed or seedling treatments of bioagents (*Trichoderma*) resulted in plant growth promotion, yield and also reduce disease severity (Singh *et al*., 2013; Jyotika Purohit *et al*., 2013). Beside this many advantages, identification of antagonistic *Trichoderma* spp is a major constraint due to variability in nature. Therefore the present investigation was undertaken with the objective of to study the cultural, morphological and variability of *Trichoderma* isolates and also to screen their capability to produce or secret the hydrolytic enzymes from the *Trichoderma* isolates of rhizosphere regions of different cropping systems in Chittoor district of Andhra Pradesh.

MATERIALS AND METHODS

Isolation and identification of native antagonistic *Trichoderma* spp. from rhizosphere of groundnut, redgram and tomato

A total of twenty seven rhizosphere soil samples were collected from rhizosphere of healthy plants in groundnut, redgram and tomato fields and shade dried. Serial dilution technique (Johnson and Curl, 1972 and Dhingra and Sinclair, 1995) was used to isolate *Trichoderma* spp**.** from rhizosphere of groundnut, redgram, tomato. Antagonistic mycoflora were isolated using Trichoderma Specific Media (TSM) of modified

methods of Saha and Pan (1997). The plates were incubated at 28 + 1°C and observed at frequent intervals for the development of colonies. Three days old colonies of *Trichoderma* were picked up and purified by single hyphal tip method.

Cultural, morphological and microscopic characterization

All the nine isolates of *Trichoderma* spp. were subjected to cultural and morphological characterization. For microscopic study, lactophenol blue staining procedure was used for proper visualization of characteristic features. The observations on growth rate, colony color, colony mycelia, colony reverse, conidiophores branching, condition, conidial color, conidia shape, conidiophores production, spore ball, sterile appendages, phialides shapes, phialides alignment and smell were recorded by following the methods of Gams and Bissett, 1998, Lieckfeidt *et al*., 2001 and Someshwar and Sitansu, 2010).

Biochemical characterization

Quantitative estimation of extracellular enzymes of test antagonist

All the isolates of *Trichoderma* spp. were biochemically characterized with respect to production of extracellular proteins and cellulytic enzymes such as chitinase, β-1, 3 glucanase and cellulase.

Chitinase assay

Chitinase assay was carried out according to the method of Reissing *et al*. (1955) with minor modifications. 200 µL reaction mixtures was prepared, which contain chitosan (1%) in the form of colloidal chitin and 20 µg of crude enzyme in 0.1 M acetate buffer. It was incubated for 24 h in water bath shaker (100rpm) at 37ºC. The reaction was stopped by boiling at 100°C and then centrifuged at 10,000 rpm for 10 min. The supernatant was used for assay by adding 33 µL of borate buffer to 166 µl of the supernatant and heating in a boiling water bath for 3 min followed by rapid cooling under tap water. One ml of DMAB (D-methyl amino benzaldehyde) reagent was added to these tubes and incubated at 37°C in water bath shaker (100 rpm) for 20 minutes and immediately the absorbance was read at 585 nm (Almeida *et al*. 2007). Comparisons were made among *Trichoderma* spp. based on absorbance read at 585 nm.

β-1, 3 glucanase assay

β-1, 3 glucanase assay was carried out by estimating the glucose released from laminarin (Miller *et al*., 1959). The reaction mixture contained 20 µg of crude enzyme in 400 µl of 0.1 M acetate buffer (pH 5.0) and 100 µL of 1% laminarin. Total volume of the reaction mixture was 500 µl. The reaction was carried out at 37°C in a water bath shaker (100 rpm) for 24 hrs. After incubation, 3 ml of DNS reagent was added to the reaction mixture and boiled for 5 min in a boiling water bath followed by cooling under running tap water(Almeida *et al*., 2007). Finally the absorbance was read at 640 nm. Comparisons were made among *Trichoderma* spp. based on absorbance read at 640 nm.

Cellulase assay

Reaction mixture containing 1ml of 1% cellulose, 2 ml of 0.05M citrate buffer (p^H 4.8) and 1ml of culture filtrate was incubated for 30 minutes at 55°C in water bath with periodical shaking and reaction was completed by boiling. The amount of glucose released in reaction was estimated by dinitrosalicyclic acid (DNSA) reagent method and enzyme activity was expressed as released of n mol^{-1} glucose Ml^{-1} min^{-1} for one unit (Bhagath and Pan, 2008).

RESULTS AND DISCUSSION

The present investigations were undertaken to characterize *Trichoderma* spp. isolated from rhizospere region of different cropping systems, morphologically with respect to growth rate, colony characters, conidiophores branching, conidiation, conidial color, conidia shape and biochemically with respect to production of enzymes like chitinase, β-1,3 glucanase and cellulase. In present study, a total of nine *Trichoderma* isolates were obtained from 27 rhizosphere samples collected from groundnut, redgram and tomato. The isolates were designated as GRT-1 to GRT-5 for the five isolates of *Trichoderma* collected from groundnut rhizosphere soils, RRT-1 to RRT-2 for the two isolates collected from redgarm and TRT-1 to TRT-2 for the two isolates collected from tomato crop. Depending on the growth rate on PDA medium, nine isolates of *Trichoderma* spp. were categorized into three groups, *viz*., very fast, fast, and medium in radial growth. It is evident from Table 1 that the isolates GRT-2, GRT-3, TRT-2, RRT-2 with 90 mm diameter radial growth within 48 h were categorized as very fast growing and the isolates GRT-1, RRT-1, TRT-1 which exhibited 90 mm in diameter within 72 h were categorized as fast growing, whereas the isolates GRT-4 and GRT-5 exhibited 90 mm in diameter within 96h were categorized as medium growing isolates (Table 1and Fig1). In similar study several scientists reported the isolation of *Trichoderma* spp. from rhizosphere

Table 1: Radial growth of *Trichoderma* isolates on PDA medium at different intervals of time

S. No.	Isolates	Colony diameter (mm) after hours				Type of growth
		24 hr	48 hr	72 hr	96 hr	
1	GRT- 1	30	67	90	-	Fast
2	GRT- 2	52	90	-	-	Very fast
3	GRT- 3	53	90	-	-	Very fast
4	GRT- 4	31	56	85	90	Medium
5	GRT- 5	33	59	84	90	Medium
6	RRT- 1	30	68	90	-	Fast
7	RRT- 2	52	90	-	-	Very Fast
8	TRT- 1	43	88	90	-	Fast
9	TRT- 2	54	90	-	-	Very Fast

GRT = Groundnut Rhizosphere *Trichoderma,* RRT = Redgram Rhizosphere *Trichoderma,*TRT = Tomato Rhizosphere *Trichoderma*

Table 2: Comparative account of morphological characters of *Trichoderma* spp. on PDA

S.No	Isolate	Surface colony colour	Colony reverse colour	Sporulation pattern
1	GRT-1	Dark green	Colour less	Alternate white and dark green concentric pattern
2	GRT-2	Dark green	Colour less	Alternate white and dark green concentric rings
3	GRT-3	Dark green	Pale Yellow	Dark green sporulation with white pustules
4	GRT-4	Dark green	Colour less	Alternate translucent and dark green concentric rings
5	GRT-5	Dark green	Colour less	Alternate dark green and light green concentric rings
6	RRT-1	Dark green	Colour less	Alternate white and dark green concentric rings
7	RRT-2	White to light green	Colour less	Green sporulation with pale green pustules
8	TRT-1	Dark green	Colour less	Alternate white and dark green concentric rings
9	TRT-2	Dark green	Colour less	White, dark green and translucent concentric pattern

Table 3: Key morphological description used for characterization of *Trichoderma* isolates

S.No	Isolate	Conidio sphore branching	Conidia shape	Chlamydo spore formation	Phialide shape grouping	Phialide	Sterile appendages	Spore balls
1	GRT-1	Long,Highly and paired branching	ellipsoidal	Infrequent and intercalary	Flask shaped	Divergent	Present	Absent
2	GRT-2	Infrequent branching	subglobose	Abundant, intercalary and terminal	Flask shape	Convergent	Absent	Present
3	GRT-3	Sparingly branched	Ellipsoidal to globose	Abundant, intercalary andterminal	Flask shaped	Convergent	Absent	Present
4	GRT-4	Sparingly branched	globose	Abundant, intercalary	Flask shaped	Convergent	Absent	Present
5	GRT-5	sparingly branched	globose	Infrequent	Flask shaped	Convergent	Absent	Present
6	RRT-1	sparingly branched	globose	Abundant, intercalary	Cylindrical	Divergent	Absent	Absent
7	RRT-2	Sparingly branched	globose	Infrequent	Flask shaped	Convergent	Absent	Present
8	TRT-1	sparingly or infrequently branched	globose	Abundant, intercalary	Flask shaped	Divergent	Present	Present
9	TRT-2	sparingly branched	globose	Abundant, intercalary and terminal	Flask shaped	Convergent	Absent	Present

Table 4: Taxonomic identification of *Trichoderma* isolates up to species level based on Morphological and microscopic characteristics

S. No	Isolate	*Trichoderma* spp.
1	GRT-1	*Trichoderrma longibrachiatum*
2	GRT-2	*Trichoderma virens*
3	GRT-3	*Trichoderma harzianum*
4	GRT-4	*Trichoderma virens*
5	GRT-5	*Trichoderma virens*
6	RRT-1	*Trichoderma harzianum*
7	RRT-2	*Trichoderma asperellum*
8	TRT-1	*Trichoderma pseudokoningii*
9	TRT-2	*Trichoderma asperellum*

soils of different crops. Sundaramoorthy and Balabaskar (2013) isolated fungal native antagonists from tomato rhizosphere soils by serial dilution technique using *Trichoderma* selective medium and identified as *T. hamatum, T. harzianum, T. koningi, T. longiconis* and *T. viride*. Cook and Baker (1983) also recorded similar observations regarding growth rate. Singh *et al.* (2006) obtained twenty seven isolates of *T. harzianum* from soil samples collected randomly from fallow agricultural fields throughout the Punjab and studied their growth rate on PDA medium and were categorised as medium, fast and very fast growing.

The growth pattern of *Trichoderma* isolates after four days of incubation at 25 + 2°C on PDA showed significant differences in nature of culture growth, surface colony color and sporulation pattern as described in Table 2. The colony color changes from white, light green to dark green with production of conidia. The conidia shapes were cylindrical, elliptical, globose to subglobse. Colony reverse was found to pale yellow in GRT-3 while remaining all isolates was colorless (Table 2). The same findings like pale or yellowish colour of reverse of colonies, rapid growth were recorded by Samuels *et al.* (2002a). Lunge and Patil (2012) studied morphological, cultural and microscopic characteristics of isolated *Trichoderma* spp. and reported similar results as observed in present study. Srivastava *et al.* (2012), Muthukumar and Pratibha Sharma (2011) used morphological description for characterization and grouping of *Trichoderma* isolates and similar observations on phenotypic characters (colony characters) showed no difference from those made earlier by Rifai (1969), Martha (1992), Mazumdar (1993) and Nagamani *et al.* (2006). Species-level identification of *Trichoderma* isolates was done based on the colour of the colony, formation of chlamydospores, branching of conidiophores, shape and disposition of phialides, shape of conidia as the main characters to identify the species. Taxonomic identification of nine isolates of *Trichoderma* spp. up to species level were done based on colony morphology and microscopic observation was done by using Labomed LX 400 microscope

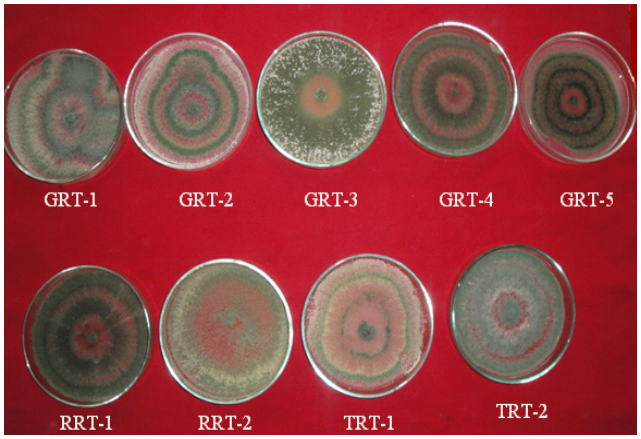
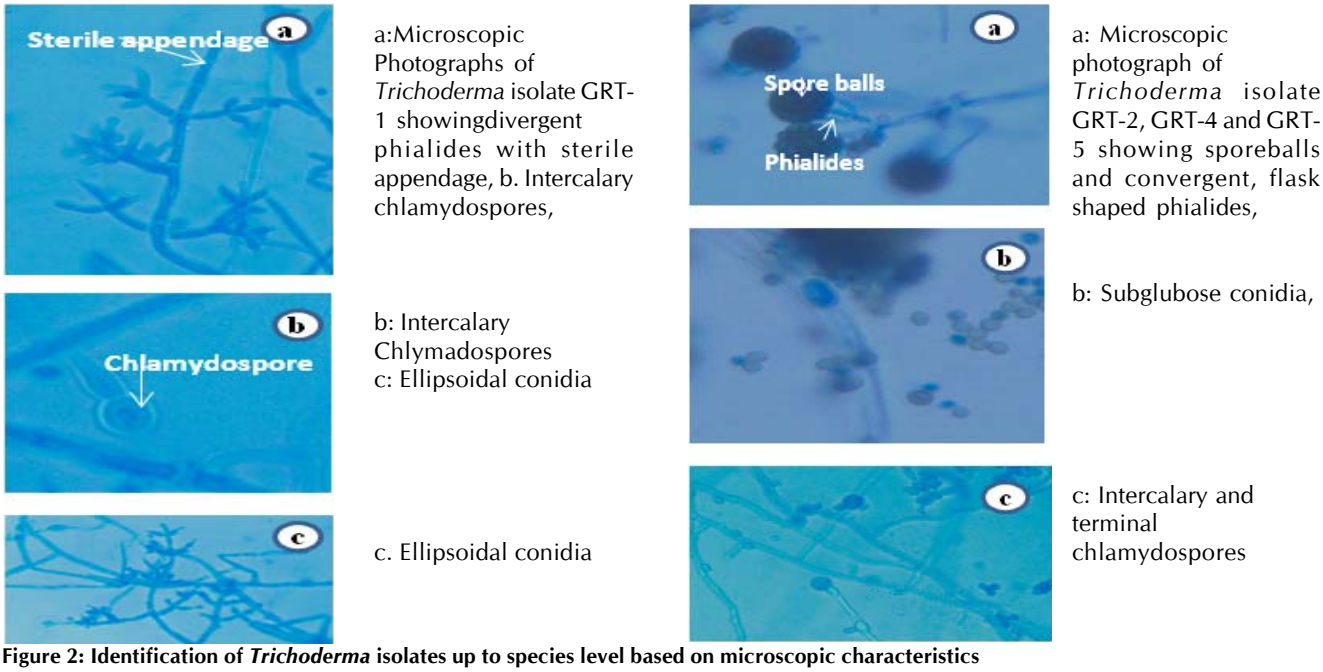


Figure 1: Pure cultures of *Trichoderma* isolates from rhizosphere region of groundnut, tomato and redgram.

Table 5: Optical density values of chitinase, β-1,3 glucanase and cellulase produced by different isolates of *Trichoderma*

S.No.	Isolate	Chitinase (OD value)	β-1, 3 Glucanase (OD value)	Cellulase(OD value)
1	GRT-1	0.225	0.083	0.194
2	GRT-2	0.199	0.081	0.233
3	GRT-3	0.290	0.130	0.260
4	GRT-4	0.190	0.079	0.159
5	GRT-5	0.180	0.080	0.156
6	RRT-1	0.189	0.089	0.159
7	RRT-2	0.282	0.096	0.241
8	TRT-1	0.201	0.085	0.244
9	TRT-2	0.280	0.100	0.257

OD values measured as absorbance.



a:Microscopic Photographs of *Trichoderma* isolate GRT-1 showingdivergent phialides with sterile appendage, b. Intercalary chlamydospores,

b: Intercalary Chlymadospores
c: Ellipsoidal conidia

c. Ellipsoidal conidia

a: Microscopic photograph of *Trichoderma* isolate GRT-2, GRT-4 and GRT-5 showing sporeballs and convergent, flask shaped phialides,

b: Subglubose conidia,

c: Intercalary and terminal chlamydospores

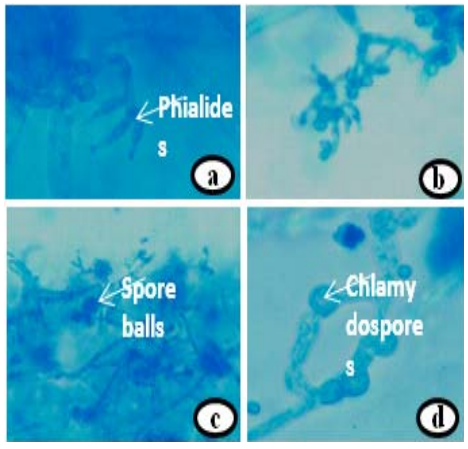
Figure 2: Identification of *Trichoderma* isolates up to species level based on microscopic characteristics

(Table 3 and 4, Fig. 2). However, in the present study, *Trichoderma* isolates could be classified here into different groups on the basis of description and keys given by Gams and Bissett 1998; Rahman *et al.*, 2011. Isolate GRT-1 showed dark green colony color, sporulation was in zonation and colony reverse was colorless. Conidiophore was long and showed highly and paired branching. Phialides with constriction and disposed in divergent verticels terminally on branches and were flask shaped. Shape of conidia was ellipsoidal. Sterile appendages were present. Chlamydospores were infrequent and intercalary. Based on these features this isolate was identified as *Trichoderma longibrachiatum*. Isolates GRT-2, GRT-4, GRT-5 showed dark green sporulation and sporulation was in zonation and colony reverse was colorless. Conidiation was in pustules with infrequent branching of conidiophores. Phialides disposed in convergent verticels terminally on branches and were flask shaped. Shape of conidia was subglobose and spores were arranged in balls. Sterile appendages were absent. Chlamydospores were frequent, intercalary and terminal. Based on these features these isolates was identified as *Trichoderma virens*. Isolates GRT-3 and RRT-1 showed dark green sporulation. Conidiation was with white pustules. Colony reverse was in pale yellow colour. Conidiophore branched sparingly. Phialides disposed in convergent verticels terminally on branches and were flask shaped. Shape of conidia was ellipsoidal to globose and spores were arranged in balls. Sterile appendages were absent. Chlamydospores were frequent, intercalary and terminal. Based on these features these isolates was identified as *Trichoderma harzianum*. Isolate TRT-1 showed dark green sporulation and sporulation was in zonation and colony reverse was colorless. Conidiophore branched sparingly or infrequently. Phialides disposed in divergent verticels terminally on branches and were flask shaped. Shape of conidia was globose and spores were arranged in balls. Sterile appendages were present. Chlamydospores were frequent and intercalary. Based on these features this isolate was identified as *Trichoderma pseudokoningii*. Isolates TRT-2 and RRT-2 showed white to light green sporulation and sporulation was in zonation and colony reverse was colorless. Conidiation was in pustules. Conidiophore branched sparingly. Phialides disposed in convergent verticels terminally on branches and were flask shaped. Shape of conidia was globose. Sterile appendages were absent. Chlamydospores were infrequent

a: Microscopic photographs of *Trichoderma* isolate GRT-3 and RRT-1 showing convergent cylindrical phialides,

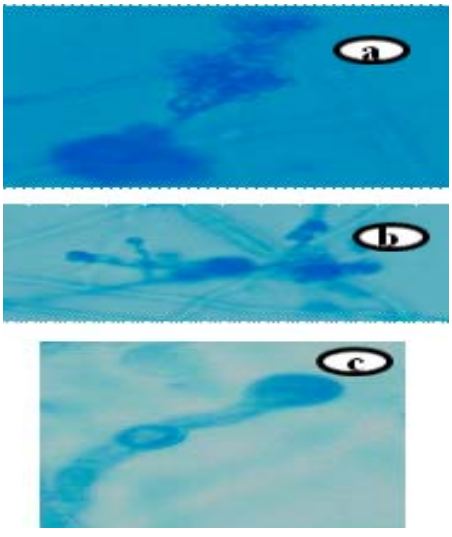
b: Intercalary and terminal chlamydospores,

c: Sporeball



a:Microscopic photographs of *Trichoderma* isolate TRT-1 showing divergent phialides

b:Globose conidia



a:Microscopic photographs of *Trichoderma* isolate RRT-2 and TRT-2 showing sporeballs and convergent, flask shaped phialides,

b:Globose conidia,

c: Intercalary and terminal chlamydospore

Figure 2: Identification of *Trichoderma* isolates up to species level based on microscopic characteristics

or not formed. Spores were arranged in balls. Based on these features they were identified as *Trichoderma asperellum* (*T. viride*) (Fig 2). All these descriptions are in agreement with the findings of earlier investigators (Gams and Bissett, 1998, Samuels *et al.*,2002, Choi *et al.*, 2003, and Shah *et al.*, 2012 who recorded the similar observations. Devi and Sinha (2014) characterized *Trichoderma* spp. from the rhizosphere soil of french bean from different locations of Manipur based on cultural and anamorphic characters *viz.*, size of phialides, phialospore and conidiophores of all *Trichoderma* spp.

All the isolates of *Trichoderma* were estimated for the production of cell wall degrading enzyme, *i.e.* chitinase assay, β-1, 3 glucanase assay and cellulase assay studied and measured in terms of optical density values (OD measured as absorbance) and presented in Table 5. In chitinase assay studies, isolate GRT-3 recorded highest optical density (0.290) followed by RRT-2 and TRT-2 which showed OD value 0.282, 0.280 respectively. In β-1, 3 glucanase assay, isolate GRT-3 recorded highest Optical density value (0.130) followed by TRT-2, RRT-2 and RRT-1 with 0.100, 0.096, 0.086 respectively. Similarly in cellulase assay, isolate GRT-3 recorded highest Optical density value (0.260) followed by TRT-2, TRT-1 and RRT-2 with 0.257, 0.244, 0.241 respectively. Mycoparasites produce cell wall degrading enzymes which allow them to bore holes into its fungal host and extract nutrients for their own growth. Most phytopathogenic fungi have cell wall that contain chitin as a structural backbone arranged in regularly ordered layers and β-1, 3-glucan as a filling material arranged in an amorphic manner. Chitinases and β-1, 3-glucanases have been found to be directly involved in the mycoparasitism interaction between *Trichoderma* spp. and its hosts (Kubicek *et al.* 2001).

The present study is in confirmation with the findings of Satyavani and Satyaprasad (2009) as they screened four isolates of *Trichoderma* spp (*T.harzianum, T.viride1, Tv2* and *Tv3*) for the production of Chitinase and β-1, 3 glucanase enzymes. Isolates of *T. harzianum* produced more chitinase and β-1, 3 glucanase than that by *T. viride* isolates. Gajera *et al.* (2012) studied the specific activities of four enzymes of *Trichoderma* spp. using cell wall of pathogen *M. phaseolina* as substrate.The release of chitinase and β-1, 3-glucanase was higher in *T. koningi* MTCC 796 followed by *T. harzianum* NBAII Th 1 and *T. hamatum* NABII Tha 1 than other *Trichoderma* spp.

REFERENCES

Almeida, F. B. Cerqueira, F. M., Silva, R. N., Ulhoa, C. J. and Lima, A. L. 2007. Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. *Biotechnology Letter*. **29:** 1189-1193.

Bisset, J. 1991a. A revision of the genus *Trichoderma* II Infragenric classification. *Canadian J. Botany*. **69:** 2357-2417.

Bisset, J. 1991b. A revision of the genus *Trichoderma* III Section. *Pachybasium*. *Canadian J. Botany*. **69:** 2373-2417.

Bisset, J. 1991c. A revision of the genus *Trichoderma* III Additional notes on section *Longibrachium*. *Canadian J. Botany*. **69:** 2418-2420.

Cook, R. J. and Baker, K. F. 1983. The nature and practice of biological control of plant pathogens. St. Paul Minnesota. *American*

Phytopathological Society. 539.

Choi, In-Young, Hong, S. B. and Yadav, M. C. 2003. Molecular and morphological characterization of green mold, *Trichoderma* spp. isolated from oyster mushrooms. *Mycobiology*. **31(2):** 74-80.

Dhingra, O. P. and Sinclair, J. B. 1995. Basic Plant Pathology methods, 2nd edn. *CRC press, Bocca Raton, America*.

Devi, Y. R. and Sinha, B. 2014. Cultural and anamorphic characterization of *Trichoderma* isolates isolated from rhizosphere of french bean (*Phaseolus vulgaris* l.) growing areas of manipur. *The Bioscan*. **9(3):** 1217-1220.

Gams, W. and Bissett, J. 1998. Morphology and identification of *Trichoderma*. *Applied Environmental Microbiology*. **65:** 3-34.

Gajera, H. P., Bambharolia, R. P., Patel, S. V., Khatrani, T. J. and Goalkiya, B. A. 2012. Antagonism of *Trichoderma* spp. against *Macrophomina phaseolina*: evaluation of coiling and cell wall degrading enzymatic activities. *J. Plant Pathology and Microbiology*. **3:** 149.

Johnson, L. F. and Curl, E. A. 1972. Methods for research on the ecology of soil borne plant pathogens. *Burgess Publishing Company*. Minneapolis. pp. 27-35.

Jyotika Purohit, Yogendra Singh, Sunaina, Bisht and Srinivasaraghvan, A. 2013. Evaluation of antagonistic potential of *Trichoderma harzianum* and *Pseudomonas fluorescens* isolates against *Gloeocercospora sorghi* causing zonate leaf spot of sorghum. *The Bioscan*. **8(4):** 1327-1330.

Kubicek, C. P., Mach, R. L., Peterbauer, C. K. and Lorito, M. 2001. *Trichoderma*: from genes to biocontrol. *J. Plant Pathology*. **83:** 11-24.

Kushwaha, M. and Verma, A. K. 2014. Antagonistic activity of *Trichoderma* Spp. (a biocontrol agent) against isolated and identified plant pathogens. *International J. Clinical and Biological Sciences*. **1:** 1-6.

Lieckfeidt, E., Kulling, C. M., Kubicek, C. P., Samuels, G. J. and Borner, T. 2001. *Trichoderma aereoviride*; Phylogenetic position and characterization. *Mycology Research*. **103:** 313-322.

Lunge, A. G. and Patil, A. S. 2012. Characterization of efficient chitinolytic enzyme producing *Trichoderma* species: a tool for better antagonistic approach. *International J. Science, Environment and Technology*. **1(5):** 377-385.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*. **31:** 426-428.

Martha, P. K. 1992. Influence of some physico-chemical factors on the germination and growth of biotype *Trichoderma harzianum* and *Gliocladium virens* M.Sc. dissertation, B.C.K.V., Mohanpur.

Mazumdar, D. 1993. Hyperparasitic potential of a few biotypes of *Trichoderma harzianum* and *Gliocladium virens* against two major pathogens of betelvine (*Piper betle* L.) M.Sc. dissertation, B.C.K.V., Mohanpur.

Muthukumar, A. and Prathibha Sharma. 2011. Molecular and morphological characters: An appurtenance for antagonism in *Trichoderma* spp. *African J. Biotechnology*. **10(22):** 4532-4543.

Nagamani, A., Kunwar, I. K. and Manoharachary, C. 2006. Handbook of soil fungi, I. K. International Private Limited, New Delhi, pp. 409-425.

Pan, S. and Bhagat, S. 2008. Characterization of antagonistic potential of *Trichoderma* sp. against some soil borne plant pathogens. *J. Biological Control*. **22(1):** 43-49.

Reissing, J. L., Strominger, J. L. and Leloir, L. F. 1955. A modified calorimetric method for the estimation of N- acetyl sugars. *J. Biological Chemistry*. 959-962.

Rifai, M. A.1969. A revision of the genus *Trichoderma*. *Mycological Papers*. **116:** 1-56.

Rahman, A., Begum, M. F., Rahman, M., Bari, M. A., Ilias, G. N. M. and Alam, M. F. 2011. Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. *Turkish J. Biology*. **35:** 183-194.

Saha, D. K. and Pan, S. 1997. Qualitative evaluation of some specific media of *Trichoderma* and *Gliocladium* and their possible modifications. *J. Mycology and Plant Pathology*. **34**: 7-13.

Samuels, G. J., Dodd, S. L., Gams, W., Castleburry, L. A. and Petrini, O. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia*. **94**: 146-170.

Singh, H. B. 2006. Management of plant pathogens with microorganisms. *J. Mycology and Plant Pathology*. **36:** 373-384.

Singh, S. K., Sharma, V. P., Sharma, S. R., Kumar, S. and Tiwari, M. 2006. Molecular characterization of *Trichoderma* taxa causing green mould disease in edible mushrooms. *Current Science*. **90(3):** 427-431.

Satyavani and Satyaprasad. 2009. Chitinase and â-1,3glucanase Production by *Trichoderma harzianum and T. viride*. *J. Mycology and Plant Pathology*. **39(1):** 144-147.

Someshwar Bhagat and Sitansu Pan. 2010. Cultural and phenotypic characterization of *Trichoderma* spp from Andaman and Nicobar Islands. *J. Mycology and Plant Pathology*. **40(1)**: 145-157.

Shah, S., Nasreen, S. and Sheikh, P. A. 2012. Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. In Kashmir. *Research J. Microbiology*. **7:** 139-144.

Singh, S. P., Singh, H. B. and Singh, D. K. 2013. *Trichoderma harzianum* and *Pseudomonas sp* mediated management of *Sclerotium rolfsii* rot in tomato (*Lycopersicon esculentum* Mill.). *The Bioscan*. **8(3)**: 801-804.

Sundaramoorthy, S. and Balabaskar, P. 2013. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici*. *J. Applied Biology and Biotechnology*. **1(3):** 36-40.

Sharma, K. K. and Singh, U. S. 2014. Cultural and morphological characterization of rhizospheric isolates of fungal antagonist *Trichoderma*. *J. Applied and Natural Science* **6(2):** 451-456.