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Study of morphology and mycoparasitism of some antagonists of *Trichoderma sp* from West Bengal, India

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Abstract:

Trichoderma sp are free living that are frequent in soil and root eco system. They can contend with other organisms. Their colonies grow fast and containing tufted or pustulate, repeatedly branched conidiophores with lageniform phialides and also bearing green conidia. Rhizoctonia solani and Sclerotium rolfsii causes solemn diseases like sheath blight in rice, damping off in chilli and collar or stem rot diseases in groundnut. A total of fourteen different Trichoderma sp denoted as T1-T14 *were isolated from rhizosphere of rice, chilli and groundnut on Potato Dextrose Agar (PDA) and Trichoderma Specific Medium (TSM). The micrometric measurement of phialide length (Average) and spore size (Average) was maximum in T1, T11 and T4 isolates as compared to other isolates. All isolates had been evidence for prospective antagonistic activity against all the tested fungal pathogens viz. Rhizoctonia solani(RS) and Sclerotium rolfsii(SR). The results portrayed in our study that T4 and T11 showed signs of strong antagonism against R. solani and T11 showing superior antagonistic effect against S.rolfsii after four days of incubation in dual culture. The calculated vigor index was also recorded maximum for the isolate T4 (1592). Two mylotic enzyme activity viz β-1,3 glucanase and chitinase of Trichoderma sp isolates were significantly positively correlated with the antagonistic activity of Trichoderma sp isolates.. Based on all the uniqueness of morphological miscellany of Trichoderma sp isolate, antagonistic scenery and cell wall degrading enzyme activity, Trichoderma sp isolates can be consigned to intend impending contender for enlargement in agriculture sectors.*

Keywords: *Trichoderma sp,* Bell's Scale, Mycolytic enzymes.

Introduction

Rice, chilli and groundnut are one of the most widely produced and consumed crops in the world and known as protective food both because of their special nutritive value and also because of their wide spread production. *Trichoderma sp* take part in

major tasks as biocontrol driving force, yet to be settled to their competence of ameliorating crop-yields by numerous roles, such as biopesticide, bioherbicides and plant growth promotion. Saleable produced *Trichoderma sp* are being used to thwart expansion of quite a lot of soils pathogenic fungi and could be a very much acquainted as resourceful biocontrol agent. Different mechanisms have been recommended as being conscientious for their bio-control commotion, which comprise competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds. They are becoming widely used in agriculture, and the most useful strains show a property that is known as 'rhizosphere competence' that is, the ability to colonize and grow in association with plant roots (Harman, 2000). *Trichoderma sp* are effective in control of soil or seed borne fungal diseases in several crop plants (Kubicek *et al*., 2001). *Trichoderma sp* are found to be capable of lysing mycelia of *Sclerotium rolfsii* and *Rhizoctonia solani*. *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* are the major fungi responsible for damping-off , root rot diseases (Shalini *et al*., 2007) and collar rot disease of groundnut (Thahir basha *et al*., 2012) respectively. *Trichoderma sp* can be taxonomically classified on the basis of conidiophore branching patterns with side branches, phialides and different size and texture of conidia. This type of individuality allow for the relatively easy identification of *Trichoderma* as a genus (Rifai, 1969; Papavizas, 1985). They are plentiful producers of extracellular proteins, and are best known for their ability to produce mycolytic enzymes such as cellulase, chitinase, β 1,3 glucanase that degrade cellulose, chitin and glucan (Sandhya *et al*., 2004; Kovacs *et al.,* 2004). Therefore, in the present investigation attempts were made for exploration of the hurriedly developing knowledge of the hostile mechanisms by which *Trichoderma sp* endow with advantageous property to plants against pathogenic fungi.

Meterials and Methods

Isolation and identification *Trichoderma sp* isolates

The collected rhizospheric soil samples were used for isolation of *Trichoderma sp* isolates through dilution plate method in PDA and TSM (Elad *et al*., 1983) The colony forming units (cfu) were counted on the plates after 96 hours of growth at 28^0C ($\pm 2^0$C) and the morphologically different colonies from each medium were picked up and purified (Johnson and Curl, 1972). Pure cultures of *Trichoderma sp* were aseptically re-cultured from stock slants onto 9 cm Petri dishes. They were allowed to sporulate under temperature of 28±2ºC; 12 hrs darkness and 12 hrs light. Mycelium typically formed within three or four days as compact or loose tufts in shades of green or yellow or less frequently white. Yellow pigment may be secreted into the agar. All these observations were recorded especially on PDA. Radial growth of

Trichoderma sp colony on PDA was observed daily until the plate was totally covered. For microscopic examination, mycelia of 3 mm was cut from the culture and transferred onto a sterile glass slide with a drop of lacto phenol-cotton blue with the help of an inoculation needle. The slide was viewed under a light microscope. A 40 x lens was used in the examination of the conidia, conidiophores, hyphae, chlamydospores and phialides where isolates identification as *Trichoderma sp* was selected.

Pathogenic fungal cultures

The *in vitro* antagonistic activity of the *Trichoderma sp* isolates were conducted with two soil borne fungal pathogens viz. *Rhizoctonia solani* and *Sclerotium rolfsii* collected from , AICRP on vegetable crops, Directorate of research, B.C.K.V. Kalyani, West Bengal.

***In vitro* antagonistic activity of *Trichoderma sp* isolates against soil borne plant pathogens**

The antagonistic effects of the *Trichoderma sp* isolates were observed against *Rhizoctonia solani* and *Sclerotium rolfsii* by dual culture technique. The Petri plates were poured with 20 ml PDA. 4 mm disc of both pathogenic fungal isolates and antagonistic *Trichoderma sp* isolates were placed at the two opposite sides of the Petri plate to evaluate their antagonistic potentiality. The antagonistic potentialities of the *Trichoderma sp* isolates were also evaluated through Bell's scale (Bell *et al*., 1982) after different time intervals.

Study of the morphological characteristic of the *Trichoderma sp* isolates

The antagonistic fungi were cultured through slide culture method where 50 % strength of dextrose was used in the medium. After 24 hrs of growth the cultured slides stained with lacto phenol-cotton blue and observed under microscope. The phialides length and spore morphology were evaluated through micrometric method.

Vigor index determination

The rhizospheric fungal isolates were bioassayed for their ability to promote seedling growth using the method as described by International Seed Testing Association (ISTA, 1966). After seven days, germination percentage, root length and shoot length were recorded.

Cell wall enzyme degrading activity of *Trichoderma sp* isolates

Isolates were screened for Cellulase, chitinolytic, β 1, 3 glucanase activities by plating on Carboxy Methyl Cellulose (CMC) agar, chitin agar and CMC agar amended with lamanarin respectively according to Kamala and Devi (2012). The agar plates were prepared and placed a 4 mm *Trichoderma sp* discs on the middle of the agar plate and incubated at 28±2◦C for 4 days. Development of halo zone around the discs was considered as positive for cell wall degrading enzyme production.

Statistical analysis

Correlation analysis was done based on the dependable (Antagonistic activity) and independable variables (Cellulase, chitinase and β-1,3 glucanase enzyme activity) performed with the SPSS 19.0 analysis system.

Results and Discussion

A total of fourteen different antagonistic *Trichoderma sp* were isolated from rhizosphere of rice, chilli and groundnut on PDA and TSM (Table 1). The fungal antagonists obtained from different agro-ecological region of West Bengal.

Table: 1 Isolates of *Trichoderma sp* from different soil rhizosphere in West Bengal

Name of the source	Name of different isolates	Antagonistic isolates
Chilli	T1,T2,T3,T4,T5,T6,T8,T11	Highly Antagonist: T1,T2,T3,T4,T5,T11 Less Antagonist:T6,T8
Rice	T7,T9,T10,T12	Highly Antagonist: T7,T12 Less Antagonist: T9,T10
Groundnut	T13,T14	Highly Antagonist : T13,T14

Out of the fourteen numbers of fungal antagonists, four were obtained from rice and eight from chilli and two from groundnut rizosphere (Table 2). Of the different sources, chilli rhizosphere is having maximum number of fungal antagonists diversity as compared to rice and groundnut (Table 2).

Table: 2 Different native antagonistic fungal isolates present on the rhizosphere of different crops

SL.NO	Isolates	Rhizosphere	Location
1	T1	Chilli	West Midnapore
2	T2	Chilli	West Midnapore
3	T3	Chilli	Kalyani
4	T4	Chilli	Kalyani
5	T5	Chilli	Kalyani
6	T6	Chilli	Kalyani
7	T7	Rice	Purulia
8	T8	Chilli	Malda
9	T9	Rice	Malda
10	T10	Rice	Kalyani
11	T11	Groundnut	Kalyani
12	T12	Rice	Bherampur
13	T13	Chilli	Bherampur
14	T14	Groundnut	Bherampur

Differences in micro morphological characteristics of highly antagonistic *Trichoderma sp* isolates were described in Table 3. The result of assessment of the

conidia, conidiophores, hyphae, chlamydospores and phialides, isolates were identified as *Trichoderma sp* up to genus level. Colony Forming Unit (CFU) of *Trichoderma sp* was initially observed as white specks on agar which then enlarged and enclosed whole petriplate within 5 to 6 days. By this time, the white colony turned grey/green/yellowish/off-white on TSM. *Trichoderma* colonies grew hastily and readily developed their typical yellow-green colour, which aided in their identification from other soil-borne fungi (Fig.1A). All the vegetative growth of mycelium could emanate either dirty yellow or brownish yellow color pigmentation which moved towards the flipside of test tubes (Fig.1B). A conidiophore is a simple or branched fertile hypha where conidia were produced. Phialides were observed for their appearance, flask like or bowling pins-shaped. The cultures were also observed for presence of chlamydospores which were often thickened hyaline cells.

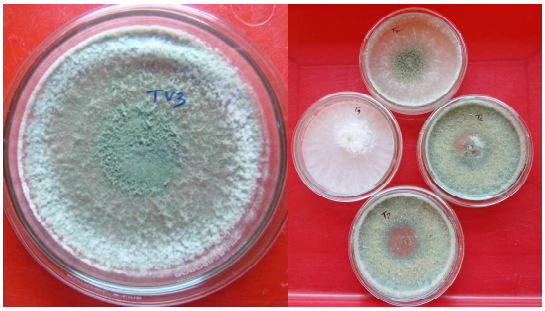


Fig: 1A *Trichoderma sp* isolates

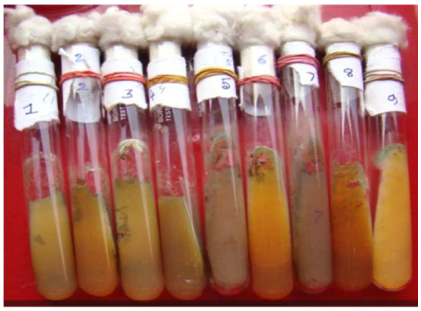


Fig: 1B *Trichoderma* sp with flip side pigmentation

Micrometric measurement of phialide length (Average) and spore size (Average) were maximum in T1 as compared to other isolates (Table 3 & Fig. 2). However, the phialide length of T2, T3 and T4 was more or less similar but the most obvious differences in spore were their size. The spore color is dark green in T1, T2, T3 whereas light green in T4 (Table 3). Based on all the characteristics the morphological diversities of *Trichoderma sp* can be attained.

Table: 3 Micrometric measurements of different morphological parameters of highly antagonist *Trichoderma* isolates

SL NO.	Isolate	Average Phialide Length(µm)	Spore	
			Size(µm)	Color
1	T1	15.818	34.673	Dark green
2	T2	6.202	4.443	Dark green
3	T3	9.308	6.586	Dark green
4	T4	12.420	11.870	Light green
5	T5	10.324	5.076	Dark Green
6	T7	7.934	4.176	Dark Green
7	T11	16.09	4.81	Dark Green
8	T12	6.054	4.123	Light Green
9	T13	7.395	6.854	Light Green
10	T14	8.651	7.032	Light Green

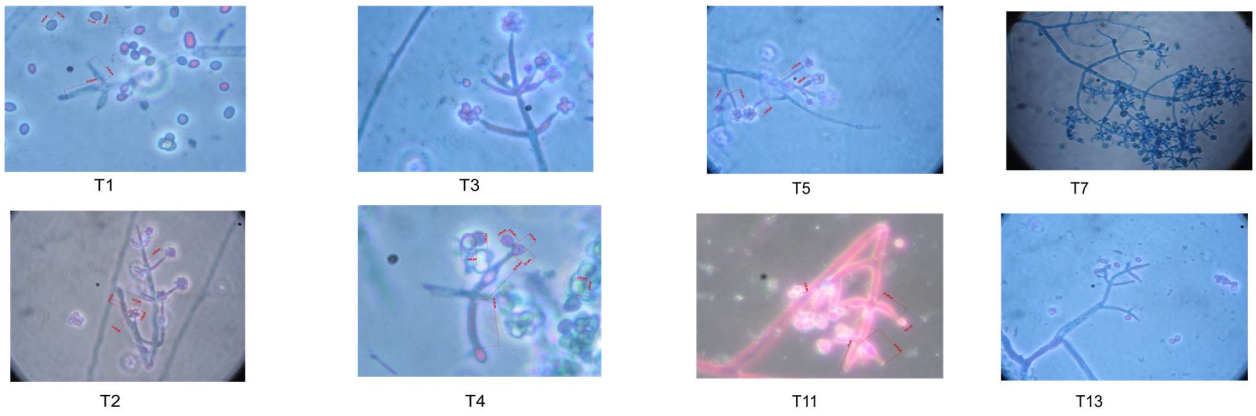


Fig: 2 Micrometric measurements of different morphological parameters of *Trichoderma* isolates

Trichoderma sp have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development. All the *Trichoderma sp* were screened for their *in vitro* antagonistic activity against soil borne fungal plant pathogens. Among these, all isolates showed potential antagonistic activity against all the tested fungal pathogens viz. *Rhizoctonia solani,* and *Sclerotium rolfsii.* The isolates T4, T5 and T11 showed evidence of strong antagonism against all the tested pathogens. All the antagonistic fungal isolates isolates showed antagonistic effect against *Rhizoctonia solani* under *in vitro* condition and inhibited the vegetative growth of the fungus at varied level. Among the isolates, T4 and T5 was found to be the

most dynamic isolate attaining S1 stage after 96 hrs of interaction. Another three isolates showing better results were T1, T3 and T13 with S1 stage attained after 120 hrs (Table 4).

Table: 4 *In vitro* antagonistic activities of the fungal isolates against soil borne plant pathogens (Using bell's Scale).

SL.NO	Isolate Vs Pathogen	Different Time Intervals					
		24Hrs	48Hrs	72Hrs	96Hrs	120Hrs	144Hrs
1	TI/RS	-	S4	S3-S2	S2	**S1**	-
2	T1/SR	-	-	S4-S3	S3	S3-S2	S2-S1
3	T2/RS	-	S4	S4-S3	S2-S3	S2	**S1**
4	T2/SR	-	S4	S4-S3	S3-S2	S3-S2	S3-S2
5	T3/RS	-	S4	S3-S2	S2	**S1**	-
6	T3/SR	-	S4	S3-S2	S2	S2	S2-S1
7	T4/RS	-	S4-S3	S2	**S1**	-	-
8	T4/SR	-	S4	S4-S3	S2-S3	S2	S2-S1
9	T5/RS	-	S4-S3	S2	**S1**	-	-
10	T5/SR	-	S4	S3-S2	S2	S2	S2-S1
11	T6/RS	-	-	S4-S3	S3-S2	S2	S2-S1
12	T6/SR	-	S4	S3	S3-S2	S2	S2
13	T7/RS	-	S4-S3	S3-S2	S2-S1	S2-S1	**S1**
14	T7/SR	-	S4-S3	S2	S2-S1	**S1**	-
15	T8/RS	-	S4	S4-S3	S2-S3	S2	**S1**
16	T8/SR	-	-	S4	S4-S3	S3	S3-S2
17	T9/RS	-	S4	S4-S3	S2-S3	S2	S2-S1
18	T9/SR	-	S4	S4-S3	S2-S3	S2	S2
19	T10/RS	-	S4	S4-S3	S3	S3	S3
20	T10/SR	-	-	S2	S2	S2	S2
21	T11/RS	-	S4	S2	**S1**	-	-
22	T11/SR	-	-	S2-S1	**S1**	-	-
23	T12/RS	-	S4	S4-S3	S3-S2	S2-S1	**S1**
24	T12/SR	-	S4	S4	S4	S4	S4
25	T13/RS	-	-	S4-S3	S3-S2	**S1**	-
26	T13/SR	-	-	S4-S3	S4-S3	S3	S3
27	T14/RS	-	-	S4	S3	S3-S2	**S1**
28	T14/SR	-	-	S4	S3-S2	S2-S1	**S1**

RS: *Rhizoctonia solani*, SR: *Sclerotium rolfsii*; Hrs: Hours; S1: 100% growth inhibition of pathogens by *Trichoderma* isolate, S2: 75% growth inhibition of pathogens by *Trichoderma* isolate, S3: 50% growth inhibition of pathogens by *Trichoderma* isolates, S4: 25% growth inhibition of pathogens by *Trichoderma* isolate.

Several workers described the coiling of *Trichoderma sp* around pathogen's hyphae and penetration of hyphae of biocontrol agent. Several researchers had shown antagonistic effect of *Trichoderma sp* against *Sclerotium rolfsii* on different hosts (Papavizas, 1985; Chet et al., 1980; Harman *et al*., 1981). Appreciably inhibition of *Drechslera triticirepentis* colony growth between 50 and 74% by *Trichoderma sp* utilizing dual culture techniques on PDA (Perello *et al*., 2003). In a similar study (Susanto *et al*., 2005) also documented that *T. harzianum* gave the highest inhibition capacity of 97.8% in dual culture analysis. *T. harzianum* had a potential biocontrol activity in a dual culture studies against the phytopathogenic fungi of *Phoma betae, Rosellinia necatrix, Botrytis cinerea* and *Fusarium oxyporum f. sp. dianthia* in the three different media (Hermosa *et al*., 2000). Devi and Reddy (2002) also reported *T. harzianum* as the most potential antagonist among the isolates of *Trichoderma sp*. Antagonistic fungi, *Trichoderma harzianum* had shown promise as a biocontrol agent of *R. solani* in chill (Bunker and Mathur, 2001). In this context, on the medium the outcome of dual culture technique exposed the speedy colonization by *Trichoderma sp* isolates. All *Trichoderma sp* isolates assessed were efficient in controlling colony growth of the soil borne plant pathogens. Evaluation of formed volatile and nonvolatile compounds also showed the adequate act on restraining mycelial development of pathogens. The results depicted here imply that from the fourteen isolates of *Trichoderma sp* used in this study, T4, T5, T11 were more accomplished of influencing the growth of all tested pathogens in dual culture (Fig. 3) and volatile and non-volatile inhibitors for isolate T2 (Fig. 4) or mycolytic enzyme activity might be key feature for restraining the growth of pathogens under controlled condition, and may be used as extensive continuum biological control agents under field condition.

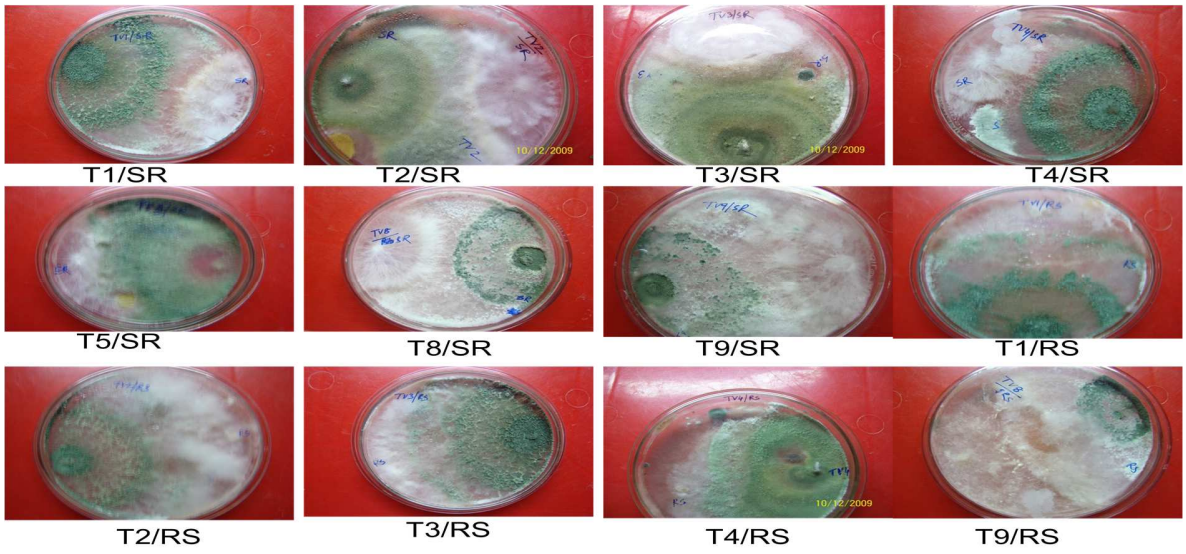


Fig: 3 Dual culture plate of *Trichoderma sp* against *Rhizoctonia solani* and *Sclerotium rolfsii*

T2/RS

Control

Fig: 4 Volatile compounds production by *Trichoderma sp* against pathogens

Groundnut seeds were treated with biotic elicitors (*Trichoderma sp*) and after seven days, the germination percentage, root length and shoot length of seedlings were recorded. Analysis of the observed data revealed that T4 isolated from chilli rhizosphere expressed best performance in plant growth promoting activity associated with seed germinability, root length and shoot length as well as vigour index in all treatments over check. Root length and shoot length of seedlings were found maximum for the isolate T4. The calculated vigor index based on germination percentage, root length and shoot length was also recorded maximum for the isolate T4 (1592) whereas contol displayed 416 (Table 5).

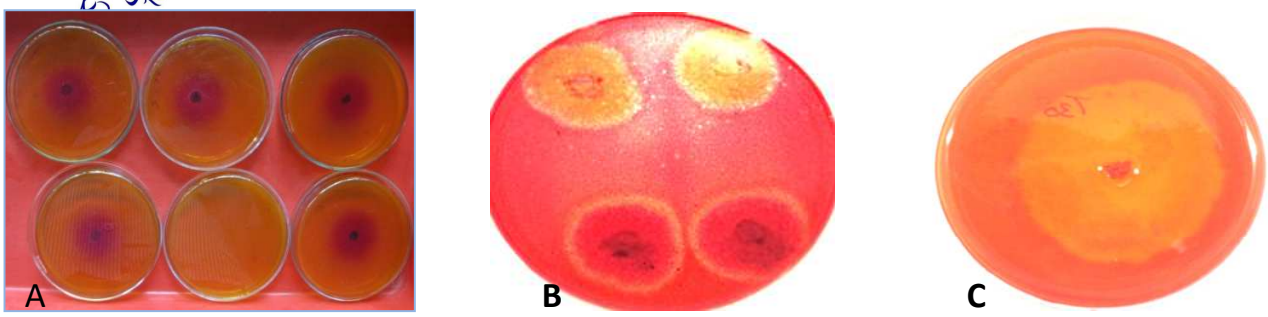
Table: 5 Effect of fungal isolates on germination %, shoot length, root length and vigor index of Groundnut (After 7 days)

Name of the antagonist	Germination %	Shoot Length (cm)	Root Length (cm)	Vigor Index
T1	85	10.5	3.1	1156
T2	75	9.8	4	1035
T3	75	13.9	5.1	1425
T4	80	14.7	5.2	1592
T5	85	12.1	4.7	1428
T6	70	8.8	2.8	812
T7	75	11.2	3.9	1132.5
T8	75	10.1	3.2	997.5
T9	70	8.3	2.6	763
T10	70	8.1	1.9	700
T11	80	10.3	4.1	1152
T12	70	9.7	2.2	833
T13	80	9.5	3.9	1072
T14	70	8.2	3.6	826
Control	65	5.3	1.1	416

Similar findings were also reported by (Yeole and Dube, 1997;Adhikari *et al*., 2013) where seed bacterization with rhizospheric fluorescent pseudomonads isolates was found to increase germination percentage, root length and shoot length of cotton, groundnut, chilli, soybean and okra. There are reports that seed bacterization with fluorescent pseudomonads and some *Bacilli sp* enhanced growth and yield of field crops like potato (Burr and Caesor, 1984; Kloepper *et al*., 1980), sugarbeets (Suslow and Schroth, 1982). The fourteen *Trichoderma sp* isolates were subjected to evaluate for their mycolytic enzyme activity. Productions of fungal cell wall degrading enzymes were analyzed because this is an important mechanism of fungal inhibition. All fourteen antagonistic rhizospheric *Trichoderma sp* isolates were found to be chitinolytic bacteria and could produced halo zones on skim milk agar that showed protease activity, production of cellulose activity on CMC (carboxy methyl cellulose) agar and exhibited β-1,3 glucanase activity, an another fungal cell wall degrading enzyme after four days of incubation. Present study clearly indicated that three isolates, viz., T4, T5 and T11 interestingly exhibited maximum all four enzyme activity. The most efficient *Trichoderma sp* T4 displayed the highest chitinolytic activity (0.400) and β 1, 3 glucanase (0.355) activity (Table 6 & Fig. 5).

Table: 6 Different Mycolytic enzyme activity of different *Tricoderma sp* isolates

***Trichoderma* Isolates**	**Cellulase activity**	**Chitinase activity**	**β-1,3Glucanase activity**
T1	0.424	0.281	0.233
T2	0.269	0.214	0.208
T3	0.324	0.303	0.281
T4	0.571	0.400	0.355
T5	0.457	0.382	0.324
T6	0.208	0.238	0.158
T7	0.471	0.258	0.292
T8	0.276	0.231	0.200
T9	0.261	0.263	0.190
T10	0.280	0.235	0.286
T11	0.516	0.333	0.308
T12	0.276	0.192	0.238
T13	0.438	0.276	0.304
T14	0.407	0.273	0.273
Control	0.424	0.281	0.233



A: Chitinase activity **B: β-1, 3 Glucanase activity** **C: Cellulose activity**

Fig: 5 Mycolytic enzymes production by *Trichoderma sp*

The two tailed Pearson's correlation between antagonistic activity of *Trichoderma sp* isolates and their cell wall degrading enzyme production revealed that β-1,3 glucanase and chitinase activity of *Trichoderma sp* isolates were significantly positively correlated with the antagonistic activity of *Trichoderma sp* isolates even at 1 % level (Table 7).

Table: 7 Correlation matrix including all the three independent variables with the dependent variable (Antagonistic activity)

Parameter	**Antagonistic activity based on Growth inhibition**	
	Correlation coefficient	Level of significance
Cellulase activity	0.366	(P=0.198)
Chitinase activity	0.786**	(P=0.001)
Beta 1,3 Glucanase activity	0.846**	(P=0.000)
Antagonistic activity	1.00	

** Significant at 1% level of probability (P=0.01).

Trichoderma sp was able to secrete hydrolytic enzymes such as chitinases, β-glucanases and cellulases and those mycolytic enzymes were responsible for lysis of the detrimental fungal cell wall (Goltapeh and Danesh, 2000) and supposed to play a basic role in the mycoparasitic activity of the fungi. The mechanism of degradation of fungal cell wall is nothing but attachment is interceded by the binding activity in between carbohydrates in the *Trichoderma sp* cell wall to lectins on the target fungus (Inber *et al*., 1996). Once in get in touch, the *Trichoderma sp* generate several fungitoxic cell-wall-degrading enzymes (Chet *et al*., 1998). The combined activities of these compounds result in

parasitism of the target fungus and dissolution of the cell walls.

Conclusion

In this study it can be concluded that the rhizospheric antagonistic fungi itself (*Trichoderma sp*) have many confirmed abilities to affect plant productivity and health positively; these can be exploited much more efficiently with a better understanding of the mechanisms and systems that operate in interactions between *Trichoderma sp* and plant pathogens. Future research will also have to be carried out to find out the worth of the application of these *Trichoderma sp* at field level.

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