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Biocontrol potential of *Trichoderma* Sp. against plant pathogens

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Abstract- Forty two strains of *Trichoderma sp.* were isolated from cultivated lands around Bangalore and analyzed for their antagonistic potential against *Sclerotium rolfsii* and *Fusarium ciceri*. The potential of biocontrol agents ultimately lies in their capacity to control pathogens i*n vivo.* Bioefficacy studies were hence conducted using chickpea (*Cicer argentums c.v. Annigeri*) as an experimental plant by the roll paper towel method. Overall the isolates T40, T35, T30 and T25 showed better antagonistic potential in addition to enhancing plant growth. The production of chitinases to break down the mycelial cell walls of fungal plant pathogens has been implicated as a major cause of biocontrol activity (Inbar and Chet, 1995). In order to study the mechanism of biocontrol, ten better performing strains were plated on media, amended with colloidal chitin and *Sclerotium rolfsii* cell wall extract. All the isolates showed chitinolytic activity on day three as well as day five. Production of endochitinase and exochitinase were assayed in liquid media using colloidal chitin amended broth. Strains T35 and T6 displayed maximum endochitinase and exochitinase activity. Although all strains exhibited cellulase activity, the quantum of enzyme produced was higher in T35 and T6. The results also indicate a positive correlation between enzyme production and bioefficacy.

Keywords- biocontrol, bioefficacy, cellulase, chitinase

Introduction

The fungal pathogens play a major role in the development of diseases on many important field and horticulture crops; resulting in severe plant yield losses. Intensified use of fungicides has resulted in accumulation of toxic compounds potentially hazardous to humans and environment and also in the buildup of resistance of the pathogens. In order to tackle these national and global problems, effective alternatives to chemical control are being employed [9]. Biological control is a nature friendly approach that uses specific microorganisms, which interfere with plant pathogens and pests to overcome the problems caused by chemical methods of plant protection. Commercial preparations of plant disease biocontrol agents are based on the practical application of rhizosphere competent species of bacteria or fungi. Although biological control occurs naturally, and is the principal reason diseases are not usually catastrophic, sufficient knowledge in many cases is not available to explain how biological control operates or how abiotic and biotic factors can be manipulated to effect the economic control of a pathogen [17]. Fungi in the genus *Trichoderma* are among the most promising biocontrol agents against plant pathogenic fungi. Specific strains have the ability to control a range of pathogens under a variety of environmental conditions. Moreover, they may be rhizosphere competent which allows them to colonize and protect plant roots. Among the action mechanisms proposed is mycoparasitism, with concomitant production of enzymes that degrade cell walls. Chitinolytic enzymes, together with ß-glucanase or cellulases are the enzymes most frequently considered critical in biocontrol. In addition, chitinolytic enzymes may be important industrially for decomposing chitinous wastes from Shellfish [11]. In spite of enormous scientific research on biological control of plant pathogens with *Trichoderma* sp., the most effective species against a wide range of pathogens is yet to be identified. With this in view, the present investigation was undertaken to examine the efficacy of selected *Trichoderma* isolates against common soil borne fungal pathogens of chickpea.

Methodology

Dual Plating

Forty two isolates of *Trichoderma* (T1 to T42) from rhizosphere soil collected around Bangalore were plated in replicates against the pathogens *Fusarium ciceri* and *Sclerotium rolfsii* to test their antagonistic potential on Potato Dextrose Agar (PDA). The colony diameters of the pathogens and the antagonists, pigmentation and overgrowth of either organism if any, were recorded periodically.

Bioefficacy (Roll Paper Towel method)

Seedling vigor testing, by the Roll Paper Towel Method (ISTA, 1976) was used for testing bioefficacy of all *Trichoderma* isolates. Pathogens *Fusarium ciceri* and *Sclerotium rofsii*, known to cause wilt, stem rot, respectively in Chickpea (*Cicer arietinum* cv. Annigeri) and other commercially important crops were grown and maintained on PDA plates. Surface disinfected seeds were first inoculated with mycelial suspension of pathogens followed by various talc preparations of *Trichoderma* isolates separately. In one treatment, fungicide (Captan at 2.5g/kg seeds) was used to treat seeds without any of the bioagents. Seeds treated with pathogen mycelium alone served as a check. For each treatment two replicates were maintained. Observations on seed germination

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and disease incidence were recorded after 10 days of incubation by following the method of Abdul-Baki and Anderson (1973) [1].

A disease grading key as described by Srivastava *et al.*, 2002 [20] was followed to rate the efficacy of bicontrol agents, based on seed rotting and infection occurring on roots and shoots.

Screening of *Trichoderma* isolates for chitin utilization

Ten strains were selected based on performance in dual Plating and *in vivo* bioefficacy. They were screened for their capacity to grow on colloidal chitin amended solid medium (CCAM) and *S. rolfsii* cell wall amended solid medium (SrCWAM). The amended media contained 0.2% colloidal chitin and *Sclerotium rolfsii* cell wall chitin respectively. The cultures were gown on Potato Dextrose Agar also for comparison. The colony growth was measured as colony diameter on the 3rd and 5th day after inoculation. For inoculation of isolates, mycelial discs of 48 hr. old cultures were used.

Assay of chitinases in liquid cultures

The crude culture filtrates of the selected ten *Trichoderma* isolates grown on colloidal chitin amended broth for seven days were used for the assay of endochitinase and exochitinase activity.

The assay mixture contained 0.5ml of 0.2% colloidal chitin, 0.5ml of enzyme solution (crude culture filtrate) and 0.5ml of 0.1M citrate buffer (pH 5.1). The reaction mixture was incubated for 4-6hrs at 37°C in a water bath. The reaction was stopped by centrifugation (5000 rpm for 10 mins). 0.5ml of the reaction mixture was taken, to which 0.1ml of 0.2M Potassium tetra borate buffer (pH 9.2) was added, followed by boiling in a water bath for 3 mins. The reaction mixture was then cooled and 5ml of p-dimethylaminebenzaldehyde (DMAB) solution was added to it. This was incubated in a waterbath at 37°C, for 20mins and cooled prior to recording absorbance. Similarly, blanks were prepared for each sample in which the samples were boiled to inactivate the enzyme activity and rest of the treatments was performed as previous. The absorbance was recorded at 585 nm.

Assay of Exochitinase

The assay mixture contained 0.5ml of 0.2M Sodium acetate buffer (pH 4.7), 0.3ml of enzyme solution (crude culture filtrate) and 0.2ml of 0.5mM p-Nitrophenyl n- acetyl β-D-glucosaminide (pNP). Blanks were prepared for each sample in which the samples were boiled to inactivate the enzyme activity and rest of the treatments was performed as previous. The reaction mixture was incubated for 4-6hrs at 37°C, in a water bath and the absorbance was read at 400nm.

Assay of Cellulases

The crude culture filtrates of the ten *Trichoderma* isolates grown on Carboxy Methyl Cellulose Amended Broth were used for the assay of Cellulase activity.

Substrate 1% carboxy methylcellulose was prepared in 0.1M Sodium Citrate buffer. To 0.45 ml of this substrate, 0.05 ml of enzyme extract (culture filtrate) was added and incubated at 55^0C for 15 minutes. To this 0.5 ml of DNS reagent was added, mixed well and boiled on a water bath (100^0C) for exactly 5 minutes. After incubation 1 ml of 40% Rochelle's salt solution was added. This was cooled to room temperature & made upto 5 ml with distilled water. Controls were made by following the same procedure as described above, except that, the tubes were boiled after adding enzyme extract to inactivate the enzyme. The intensity of yellow colour was measured at 540ηm against blank. The results were expressed as $\mu M\ ml^{-1}$ of culture filtrate.

Discussion

In recent years, research on biological control has gained momentum for controlling serious soil born plant pathogens like *Fusarium, Rizoctonia, Macrophomina, Sclerotium, Pythium and Phytophthora* spp, employing *Trichoderma* and *Gliocladium* species and varied success has been achieved.[7,19,18,14]. In the current study investigations were carried out on the forty two *Trichoderma* strains, isolated from rhizosphere soil samples from cultivated lands around Bangalore, for their biocontrol potential against the pathogens - *Sclerotium rolfsii* and *Fusarium ciceri*. All strains were subjected to dual plating on Potato Dextrose Agar, against two pathogens *viz*, *F. ciceri* and *S. rolfsii.* Observing the zone of inhibition at the point of contact of pathogen and the antagonist and measuring their colony diameter on the third and fifth day after inoculation, served as an indicator of their *in vitro* biocontrol activity. The percentage reduction of growth over control for the pathogen *F. ciceri,* was calculated and the isolate T40 (153.892%) was found to be the most effective on the third day after inoculation. On the fifth day of inoculation, T40 (186.173%) followed by T27 (152.099%) were the best performers. The lowest percentage reduction of *F. ciceri* over control was recorded by T38 on the third day (9.98%) and on the fifth day (12.346) (Table 1). Against *S. rolfsii,* T30 (66.05%) and T6 (63.19%), showed maximum antagonistic activity on the third day of incubation. On day five, maximum percentage reduction of the pathogen growth over control indicated T30 (43.22%) and T6 (40.0%) to be the best performers while T13 (-41.22%) recorded the least (Table 2). Similar antagonistic ability of *Trichoderma* isolates against the plant pathogen *S. rolfsii,* indicated by formation of lytic zones along the point of contact is corrobated by Haran

et al, 1993 [10]. The potential of biocontrol agents ultimately lies in their ability to control plant pathogens *in vivo*. Therefore, the isolates were tested for their bioefficacy *in vivo,* using Roll-Paper Towel method, with Chickpea (*Cicer arietinum* cv. Annigeri), as the experimental plant. High percentage of germination was observed in the isolates T40, T35, T30 and T25, and the disease incidence was found to be nil, compared to the pathogen check. A disease grading key, was used for rating efficacy of the bioagents used, based on the seed rotting, infection occurring on roots and shoots. All bioagent treatments and fungicide treatments recorded significantly less disease incidence, compared to the pathogen check (Tables 3 & 4). These results of improved plant growth are well supported by earlier works of Abdul-Baki and Anderson, 1973; and Srivastava *et al*, 2002[1, 20]. The mechanism of biocontrol activity is a subject of great curiosity. The production of chitinases, to breakdown the mycelial cell wall of the fungal plant pathogens have been implicated as a major cause of biocontrol activity. Both endochitinases and exochitinases have been considered to be responsible for the biocontrol activities exhibited. Inbar and Chet, 1995 indicated that the production of these enzymes by species of *Trichoderma* might be associated with their antagonistic behaviour against other soil fungi. Studies conducted by de la Cruz et al. (1993)[6]; and Lorito et al. (1996)[16] showed that the endochitinases were among the most effective for both antifungal and lytic activities when compared with other chitinolytic enzymes. On the solid media, amended with colloidal chitin and *S. rolfsii* cell wall extract, all of the strains exhibited strong chitinolytic activity, as determined by the formation of clearing zones on the third as well as the fifth day (Table 5). The results obtained indicate a positive correlation between chitinolytic activity and bioefficacy. These findings however, differ from the results obtained by Cotes et al., (1994) [5], which showed that the protective ability of *Trichoderma* isolates was unrelated to the *in vitro* enzyme activities in culture filtrates, regardless of the carbon source used. Further, endochitinase produced by isolates T35, T6 and T7 and exochtinase produced by isolates, T35, T6 and T30, also associate well with their bioefficacy [Figs. (1) & (2)]. Production of exochitinase by efficient *Trichoderma* isolates, for biocontrol of *Rhizoctonia solani*, which causes root rot of Soya bean, has been observed and documented by Bertagnolli, Dal Soglio and Sinclair (1995) [2]. The above observations indicate a strong correlation between bioefficacy and chitinase production, which is consistent with the findings of Elad et al. (1982)[8], Cook and Baker (1983) [4], Chet, I. (1987, 1990) [3], Inbar and Chet (1995) [12], Haran et al., (1996) [10], Krishnamurthy et al., (1999) [15], and Stephen-Jebakumar et al., (2000) [13]. All strains exhibited cellulolytic activity. The activity was seen to be high in T35 followed by T6. T27 being a moderate performer in dual plating and bioefficacy studies, recorded high cellulolytic activity [Fig. (3)]. However, a positive correlation was seen between the cellulolytic activity and *in vivo* bioefficacy for the isolate T35. In the present study the *Trichoderma* isolates, T35 and T6, stand out with respect to their biocontrol potential followed by T25 and T30. However, these isolates must be evaluated under the full range of conditions that are experienced by the farmers. Field trials need to be conducted across different agro-ecological zones to utilize the potential exhibited by them.

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Table 1: Dual plating of Trichoderma against F. ciceri

Culture	3rd day			5th day		
	T	Fc	Fc (% reduction over control	T	Fc	Fc (% reduction over control
T1	10.58	8.54	40.719	10.6	9.12	36.543
T2	10.57	5.09	109.381	10.64	5.2	134.321
T3	5.9	3.53	47.305	6.2	4.2	49.383
T4	6.45	4.56	37.725	6.7	4.79	47.16
T5	5.67	3.94	34.531	6.12	4.2	47.407
T6	10.18	3.95	124.351	7.76	4.1	90.37
T7	7.69	5.36	46.507	7.9	5.9	49.383
T8	8.09	6.57	30.339	8.2	6.7	37.037
T9	7.7	4.56	62.675	7.775	4.6	78.395
T10	6.8	5.4	27.944	7	5.8	29.63
T11	7.6	5.31	45.709	7.8	5.9	46.914
T12	10.56	8.6	39.122	10.6	9	39.506
T13	9.56	7.34	44.311	9.6	7.59	49.63
T14	10.73	5.81	98.204	10.8	6.38	109.12
T15	9.09	6.59	49.9	9.1	7.14	48.395
T16	7.78	6.36	28.343	7.8	6.4	34.568
T17	7.73	5.98	34.93	7.8	6.2	39.506
T18	7.23	4.84	47.705	7.74	5.65	51.605
T19	7.25	5.38	37.325	7.69	5.69	49.383
T20	7.14	3.01	82.435	7.775	3.1	115.432
T21	6.23	5.4	16.567	6.3	5.64	16.296
T22	5.48	3.21	45.309	5.56	3.64	47.407
T23	3.68	2.2	29.541	3.7	2.48	30.123
T24	4.9	3.78	22.355	5.01	3.82	29.383
T25	7.78	3.03	94.81	7.825	3.14	115.679
T26	7.89	6.72	23.35	7.92	6.9	25.185
T27	11.48	4.63	136.73	11.59	5.43	152.099
T28	9.58	8.21	27.345	9.61	8.5	27.407
T29	9.26	7.98	25.549	9.35	8.12	30.37
T30	10.95	5.14	115.968	11.02	5.26	142.222
T31	10.92	9	38.323	10.97	9.7	31.358
T32	11.32	9.56	35.13	11.45	9.87	39.012
T33	9.58	8.24	26.747	9.75	8.56	29.383
T34	8.76	7.21	30.938	8.89	7.64	30.864
T35	10.5	5.85	92.814	10.62	5.94	115.556
T36	10.23	7.79	48.703	10.35	8.42	47.654
T37	9.08	8.57	10.18	9.28	8.75	13.086
T38	9.12	8.62	9.98	9.2	8.7	12.346
T39	4.6	3.3	25.95	5	3.89	27.407
T40	12.62	4.91	153.892	12.75	5.21	186.173
T41	5.89	4.52	27.345	5.92	4.69	30.37
T42	6.56	5.32	24.751	6.68	5.8	21.728
F. ciceri-control		5.01			4.05	
SD	2.1239	1.9126		2.0627	1.9403	
SEM	1.5018	1.3524		1.4586	1.372	

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Table 2: Dual plating of Trichoderma against S. rolfsii

Culture	3rd day			5th day		
	T	Sr	Sr (% reduction over control)	T	Sr	Sr (% reduction over control)
T1	6.44	4.65	36.61	7.75	5.74	22.33
T2	7.95	5.59	48.26	7.96	5.81	23.89
T3	7.81	6.14	34.15	7.93	6.31	18
T4	8	5.58	49.49	7.65	5.2	27.22
T5	7.28	6.28	20.45	7.96	7.04	10.22
T6	7.98	4.89	63.19	8.01	4.41	40
T7	7.91	5.68	45.6	7.93	5.69	24.89
T8	8.14	5.81	47.65	8	5.48	28
T9	7.95	5.71	45.81	7.64	8.26	-6.89
T10	8.14	4.91	66.05	8.19	4.3	43.22
T11	7.18	6.64	11.04	7.2	7.6	-4.44
T12	7.88	6.73	23.52	7.94	6.53	15.67
T13	4.65	7	-48.06	4.68	8.39	-41.22
T14	7.31	5.95	27.81	7.79	6.59	13.33
T15	5.59	6.73	-23.31	5.25	8.04	-31
T16	7.36	5.94	29.04	7.23	6.41	9.11
T17	7.29	6.1	24.34	8.36	7.22	12.67
T18	7.29	7.06	4.7	6.29	7.8	-16.78
T19	7.41	6.29	22.9	7.71	6.48	13.67
T20	6.8	6.9	-2.04	9	9	0
T21	7.39	6.01	28.22086	7.42	6.2	13.55556
T22	6.5	6.43	1.431493	6.78	6.65	1.444444
T23	6.66	6.79	-2.65849	6.75	6.85	-1.11111
T24	7.56	6.65	18.60941	7.68	6.78	10
T25	7.88	6.73	23.52	7.94	6.53	15.67
T26	6.89	5.9	20.2454	6.92	6.01	10.11111
T27	7.91	5.68	45.6	7.93	5.69	24.89
T28	6.56	7.01	-9.20245	6.7	7.1	-4.44444
T29	6.77	6.8	-0.6135	6.8	6.82	-0.22222
T30	8.14	4.91	66.05	8.19	4.3	43.22
T31	7.2	6.9	6.134969	7.89	6.99	10
T32	7.6	7.3	6.134969	7.88	7.56	3.555556
T33	6.9	5.67	25.15337	7.3	6.07	13.66667
T34	7.4	7.04	7.361963	7.48	7.13	3.888889
T35	8.14	5.81	47.65	8	5.48	28
T36	8.01	7.88	2.658487	8.2	7.95	2.777778
T37	7.77	6.89	17.99591	7.99	7.12	9.666667
T38	8	7.79	4.294479	8.01	7.83	2
T39	6.92	7.2	-5.72597	6.95	7.6	-7.22222
T40	8	5.58	49.49	7.65	5.2	27.22
T41	7.07	6.92	3.067485	7.12	6.97	1.666667
T42	7.17	7.02	3.067485	7.22	7.08	1.555556
S. rolfsii control		4.89			9	
SD	0.7235	0.7741		0.7871	1.0962	
SEM	0.1116	0.1731		0.176	0.2451	

Table 3: Bioefficacy of Trichoderma isolates Vs F.ciceri - disease incidence

Treatment	% Germination	Disease Incidence %	Rating
	Mean	Study %	
T1 Vs F.c	84	4	efficient
T2 Vs F.c	95	Nil	highly efficient
T3 Vs F.c	86	8	efficient
T4 Vs F.c	78	22	moderately efficient
T5 Vs F.c	90	8	efficient
T6 Vs F.c	96	Nil	highly efficient
T7 Vs F.c	92	6	efficient
T8 Vs F.c	90	7	efficient
T9 Vs F.c	96	Nil	highly efficient
T10 Vs F.c	77	21	moderately efficient
T11 Vs F.c	79	24	moderately efficient
T12 Vs F.c	81	12	efficient
T13 Vs F.c	80	24	moderately efficient
T14 Vs F.c	96	Nil	highly efficient
T15 Vs F.c	80	20	moderately efficient
T16 Vs F.c	88	4	efficient
T17 Vs F.c	90	4	efficient
T18 Vs F.c	79	16	moderately efficient
T19 Vs F.c	83	4	efficient
T20 Vs F.c	88	4	efficient
T21 Vs F.c	87	9	efficient
T22 Vs F.c	89	8	efficient
T23 Vs F.c	82	16	moderately efficient
T24 Vs F.c	92	8	efficient
T25 Vs F.c	97	Nil	highly efficient
T26 Vs F.c	72	25	moderately efficient
T27 Vs F.c	94	Nil	highly efficient
T28 Vs F.c	81	19	moderately efficient
T29 Vs F.c	72	26	moderately efficient
T30 Vs F.c	98	Nil	highly efficient
T31 Vs F.c	71	27	moderately efficient
T32 Vs F.c	66	31	moderately inefficient
T33 Vs F.c	32	68	highly inefficient
T34 Vs F.c	79	18	moderately efficient
T35 Vs F.c	96	Nil	highly efficient
T36 Vs F.c	54	39	moderately inefficient
T37 Vs F.c	69	30	moderately efficient
T38 Vs F.c	77	9	efficient
T39 Vs F.c	80	16	moderately efficient
T40 Vs F.c	97	Nil	highly efficient
T41 Vs F.c	87	10	efficient
T42 Vs F.c	74	23	moderately efficient
F. ciceri-control		54	

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Table 4: Bioefficacy of Trichoderma isolates Vs S. rolfsii - disease incidence

Treatment	Disease Incidence %		
	Mean	Study %	
T1 Vs S.r	88	4	efficient
T2 Vs S.r	98	Nil	highly efficient
T3 Vs S.r	86	8	efficient
T4 Vs S.r	85	7	Efficient
T5 Vs S.r	89	8	Efficient
T6 Vs S.r	92	Nil	highly efficient
T7 Vs S.r	90	9	Efficient
T8 Vs S.r	85	13	Efficient
T9 Vs S.r	89	4	Efficient
T10 Vs S.r	82	15	Efficient
T11 Vs S.r	78	20	moderately efficient
T12 Vs S.r	79	18	moderately efficient
T13 Vs S.r	81	8	moderately efficient
T14 Vs S.r	96	Nil	highly efficient
T15 Vs S.r	80	20	moderately efficient
T16 Vs S.r	88	4	Efficient
T17 Vs S.r	89	4	Efficient
T18 Vs S.r	92	8	moderately efficient
T19 Vs S.r	79	20	moderately efficient
T20 Vs S.r	96	Nil	highly efficient
T21 Vs S.r	85	13	Efficient
T22 Vs S.r	85	9	Efficient
T23 Vs S.r	80	14	Efficient
T24 Vs S.r	90	10	Efficient
T25 Vs S.r	92	Nil	highly efficient
T26 Vs S.r	75	21	moderately efficient
T27 Vs S.r	92	Nil	highly efficient
T28 Vs S.r	79	20	moderately efficient
T29 Vs S.r	70	24	moderately efficient
T30 Vs S.r	96	Nil	highly efficient
T31 Vs S.r	69	31	moderately inefficient
T32 Vs S.r	68	30	moderately inefficient
T33 Vs S.r	30	70	highly inefficient
T34 Vs S.r	82	9	Efficient
T35 Vs S.r	96	Nil	highly efficient
T36 Vs S.r	49	48	Inefficient
T37 Vs S.r	70	22	moderately efficient
T38 Vs S.r	75	10	Efficient
T39 Vs S.r	84	10	Efficient
T40 Vs S.r	92	Nil	highly efficient
T41 Vs S.r	84	16	moderately efficient
T42 Vs S.r	72	25	moderately efficient
S. rolfsii-control		56	

Table 5: Growth of Trichoderma isolates on PDA, SrCWAM, CCAM study on solid media

Table 5: Growth of *Trichoderma* isolates on PDA, *Sr*CWAM, CCAM study on solid media

	3rd day observation			5th day observation		
	PDA	SrCWAM	CCAM	PDA	SrCWAM	CCAM
Treatments	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
T2	8.275	5.4875	5.65	9	8.475	8.4625
T40	8.3	5.7125	4.6875	9	8.5875	8.275
T6	6.65	5.35	5.0875	9	8.225	7.4125
T27	5.4	4.75	5.125	9	8.4625	8.4625
T35	8.275	5.475	6.4125	9	8.4875	8.4625
T9	8.3875	4.575	4.0875	9	8.55	7.4
T30	5.1375	5.45	4.525	9	8.7125	8.4375
T20	7.9625	5.4	4.2125	9	8.6625	8.1125
T25	6.275	4.625	4.2	9	8.35	7.425
T14	8.6375	4.2	5.475	9	8.375	8.3875
SD	1.3359	0.5137	0.7494	0	0.1477	0.4758
SEM	0.2987	0.1149	0.1676	0	0.033	0.1064

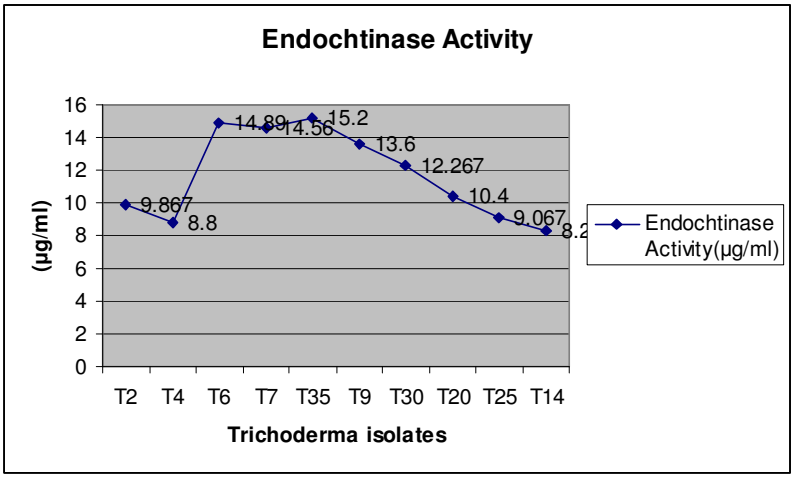

Fig. 1-

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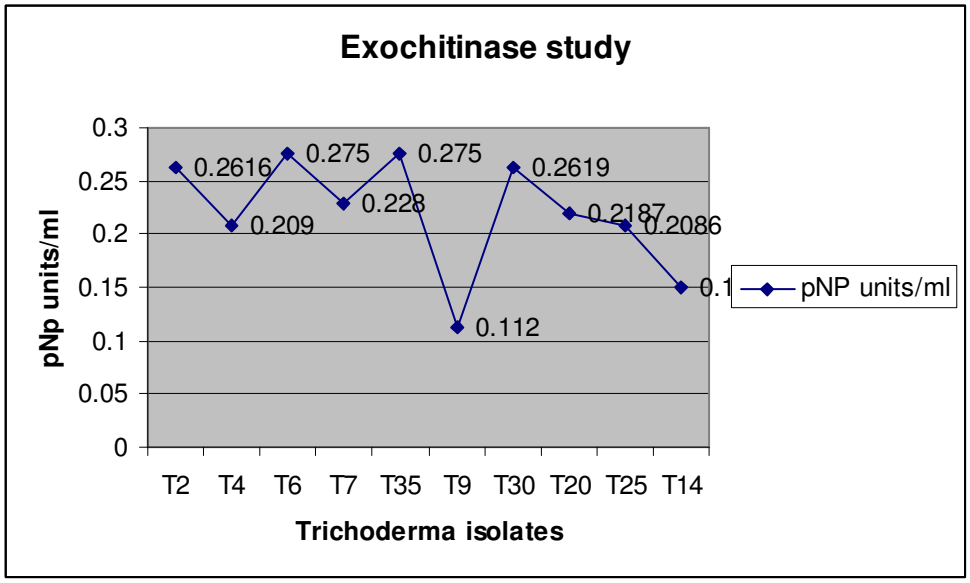
Exochitinase study
pNp units/ml
0.3
0.25
0.2
0.15
0.1
0.05
0
0.2616
0.209
0.275
0.228
0.275
0.112
0.2619
0.2187
0.2086
T2
T4
T6
T7
T35
T9
T30
T20
T25
T14
Trichoderma isolates
pNP units/ml

Fig. 2-

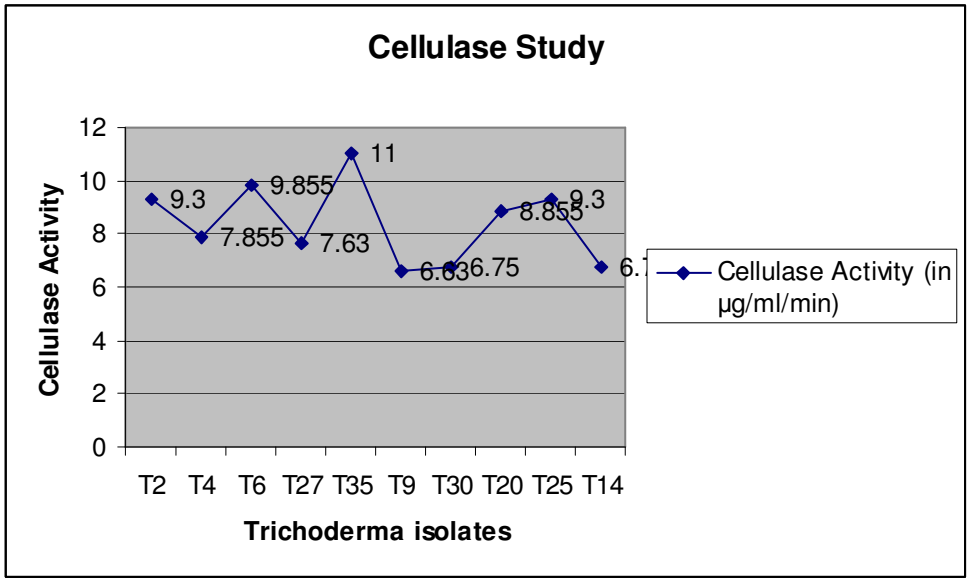
Cellulase Study
Cellulase Activity
12
10
8
6
4
2
0
9.3
7.855
9.855
7.63
11
6.63
6.75
8.855
9.3
T2
T4
T6
T27
T35
T9
T30
T20
T25
T14
Trichoderma isolates
Cellulase Activity (in µg/ml/min)

Fig. 3-