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Chitinase production ability of *Trichoderma* spp. and their antagonistic activity against soil borne plant pathogens

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Abstract
Four *Trichoderma* spp. were tested for chitinase enzyme activity and antagonistic activity against soil borne plant pathogens. All the tested four isolates of *Trichoderma* spp. were produced the chitinase enzyme and also inhibit the growth of test soil borne plant pathogens in-vitro. However highest production of chitinase enzyme was recorded by *T. asperellum* (0.65/1.02) chitinase units/mg of protein followed by *T. reesei* (0.64/0.94), *T. harzianum* (0.46/0.92) and *T. hamatum* (0.36/0.80). All the tested *Trichoderma* spp. inhibited the growth of soil-borne plant pathogens invitro. Maximum% inhibition of *Fusarium oxysporum* f. sp. *vasinfectum* was recorded in *T. reesei* i.e (75.27%). However, maximum growth inhibition of *Sclerotium rolfsii* were recorded in *T. asperellum* i.e. (67.07%) and in *Rhizoctonia bataticola* (69.84%) were recorded in *T. asperellum*.

Keywords: % - Percent, / - Per, DAI - Days after incubation

Introduction
The use of bio-agents having bio-control and plant growth promotion (PGP) activities have been considered as naturally and environmentally acceptable alternative to minimize the use of synthetic chemicals and their hazardous effects, and to provide protection to the plants against resident pathogen populations (Schipper *et al*.1987, Lugtenberg *et al*. 2001) [16, 11]. Fungi are the most extensively researched group of biological control agents. *Trichoderma* spp. produce chitinases enzyme which lysis the cell wall of plant pathogenic fungi. Chitin is the major constituent of cell wall of plant pathogenic fungus for example, *Rhizoctonia, Fussarium, Sclerotium.* Among these, several species of *Trichoderma* are well documented mycoparasites and have been used successfully against certain pathogenic fungi.

Materials and Methods
Estimation of chitinase enzyme
All the four *Trichoderma* spp. was assessed for chitinase enzyme activity by the method of Kulkarni and Ramanujan *et al*. (2010) [9]. Czapek's broth medium was used for the growth of *Trichoderma* spp. along with crab shell chitin 5 gram in 1 litre medium. The 2 fungal disc of respective *Trichoderma* spp. were inoculated in 250 ml conical flask containing 100 ml sterilized Czapek's medium aseptically. All the inoculated conical flask was incubated for 48 h at 25 $\pm$ 2 °C in BOD incubator then after 48 h of incubation, the flasks were transferred in rotary shaker at 150 rpm at 25 °C for 4 days. The culture filtrate of all *Trichoderma* spp. were collected by passing through Whatman no. 1 filter paper aseptically. The culture filtrate of all four *Trichoderma* spp. were used for further study. The enzyme solution was prepared by addition of potassium phosphate buffer with PH 6.7 in 6:1 concentration. After that, 0.5 ml culture filterate (enzyme solution) and 0.5 ml colloidal chitin were mixed in 5 ml distilled water and kept overnight. Next day absorbance was recorded at 510 nm.

Colloidal chitin preparation
Roberts and Selintrenikoff (1988) [15] method was used for the preparation of colloidal chitin
1. A 20 gram crab shell chitin powder was dissolved in 350 ml cold concentrated HCL and placed at 4^{O} C for 24 h.
2. The mixture was filtered through Whatman no. 1 filter paper aseptically.
3. Centrifugation of chitin pellets was done at 12000 rpm for 18 minutes. Then this pellets was washed by using distilled water two to three times

4. Then washed colloidal chitin/pellets was lyophilized to dryness and stored at -20 °C.

Phosphate buffer preparation

- 136 gram, Potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 1000 ml of distilled water.
- Also 174 gram, Dipotassium phosphate ($K_2 HPO_4$) was dissolved in 1000 ml of distilled water. Both the suspension were mixed together for buffer preparation and kept pH of buffer 6.7.

Standard graph preparation

Dextrose is used for preparation of standard graph as a glucose source. Glucose solution concentration i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0% was made by adding each concentration in 100 ml distilled water. From each concentration 0.5 ml of glucose solution and 0.5 ml colloidal chitin solution were mixed in 5 ml distilled water in test tubes and incubated for 24 h at 28 °C. The absorbance was recorded at 510 nm.

Protein estimation

Chemicals

1. Na_2CO_3 (Sodium carbonate) – 2 g (A)
2. NaOH (Sodium hydroxide) – 0.1 g (A)
3. Na-K (Sodium potassium tertarate) – 1 g (B)
4. $CuSO_4$ (Copper sulphate) – 0.5 g (B)
5. Folin-Phenol reagent (FC) – 1:1
6. Trichloroacetic acid (TCA) – 1 ml of 10%

Lowry *et al*. 1951 [10] method was used for protein estimation. Four *Trichoderma* spp. were grown on PDB for 7 days. Incubation after seven days, mycelial mat were collected and washed with distilled water. By using sterilized, pre-chilled liquid nitrogen the mycelial mat was crushed in mortar and pestle (Boregowda *et al*. 2017) [5]. In centrifuge tube 1ml/g sample and 1 ml of 10% TCA (Trichloroacetic acid) was added for precipitation of protein and centrifuge. Supernatant of the samples were discarded and repeated this procedure for two times.

Reagent addition Procedure

A - Take 0.1 g NaOH and dissolved in 100 ml distilled water and also add 2 g Na_2CO_3.

B – Take 1 g Na-K tertarate and dissolved in 100 ml distilled water and also add 0.5 g $CuSO_4$.

C – Take A solution 50 ml and B solution 1 ml and mixed it. (This solution is made freshly when we perform experiment).

(A + B = C Solution)

Procedure for protein estimation

- Take 6 test tube. In 5 test tube add BSA solution and 1 test tube is for blank.
- B.SA solution in test tube is 0.2, 0.4, 0.6, 0.8, 1 ml in 5 test tube. Add distilled water in B.SA solution for volume make –up, just like in 0.2 ml add 0.8 ml distilled water, in 0.4 ml add 0.6 ml distilled water and so on.
- Ater that add 4 ml reagent C in all 6 test tube including blank.
- Incubate these test tube at room temperature for 10 minutes.
- Add 0.5 ml Folin-phenol reagent (FC) in all 6 test tubes including blank.
- Incubate these tubes at room temperature for 15 to 30 minutes.
- Take a reading at Optical density (OD) 660 – 750 nm.

Dual culture technique

Antagonistic activity of four species of *Trichoderma* such as *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma reesei* and *Trichoderma hamatum* were studied against *Rhizoctonia bataticola*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *vasinfectum* by using dual culture method (Bastakoti et al. 2017) [2]. In petri plates, 5 mm mycelial disc of 7 days old culture of *Trichoderma* spp. and the soil borne pathogens were placed on the opposite ends of the plate at equal distance on same day. 5 mm disc of pathogenic fungi was also placed alone in centre of the plate. Three replications of each treatment were maintained. The plates were incubated upto 7 days, after 7 days of incubation period, % inhibition was calculated by using following formula.

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \text{ X } 100$$

Results and Discussion

Estimation of chitinase enzyme units/mg of protein in *Trichoderma* spp.

The chitinase enzyme and protein in four *Trichoderma* spp. were evaluated and the data were recorded in Table 1. *Trichoderma* spp. T_1 (*T. asperellum*) content maximum 0.65/1.02 chitinase units/mg of protein, which is significantly superior over all this three species of *Trichoderma*. The next best species T_3 (*T. reesei*) content 0.64/0.94 chitinase units/mg of protein followed by T_2 (*T. harzianum*) content 0.45/0.92 chitinase units/mg of protein. Whereas, the least amount of 0.36/0.80 chitinase units/mg of protein was recorded in T_4 (*T. hamatum*) chitinase units/mg of protein.

Table 1: Estimation of chitinase enzyme and protein in four *Trichoderma* spp.

Tr. No.	*Trichoderma* spp.	Chitinase enzyme /Protein (mg)			Mean
		T_1	T_2	T_3	
T_1	*Trichoderma asperellum*	0.65	0.72	0.59	0.65/1.02
T_2	*Trichoderma harzianum*	0.33	0.44	0.60	0.46/0.92
T_3	*Trichoderma reesei*	0.66	0.70	0.56	0.64/0.94
T_4	*Trichoderma hamatum*	0.35	0.23	0.51	0.36/0.80
S.E. ±					0.17
C.D. at 1%					0.53

Bhat *et al*. (2009) [4] recorded that differentiation in chitinolytic activity in different *Trichoderma* isolates.

Agrawal and Kotasthane (2012) [1] and Giridhar *et al*. (2012) [6] also studied the chitinase activity of *Trichoderma* spp. and reported that the purified chitinase showed antifungal activity against phytopathogenic fungi.

Efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *vasinfectum* at 7 DAI

Efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *vasinfectum* was tested by dual culture technique. Observations on per cent growth inhibition were recorded at 7 DAI (Day after Inoculation) and% inhibition of *Fusarium oxysporum* f. sp. *vasinfectum* was recorded maximum in *Trichoderma* spp. T_2 (*T. reesei*) 75.27% which was followed by T_1 (*T. asperellum*) 73.92% then T_3 (*T. harzianum*) 71.96%.

Whereas the minimum% inhibition was recorded in T_4 (*T. hamatum*) 69.32%.

Plate 1: Efficacy of four *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *vasinfectum*

Table 2: Antagonistic efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *vasinfectum* by dual culture method at 7 DAI

Tr. No.	*Trichoderma* spp.	Mean (mm)	% Inhibition
T_1	*T. asperellum*	23.47	73.92
T_2	*T. reesei*	22.25	75.27
T_3	*T. harzianum*	25.23	71.96
T_4	*T. hamatum*	27.61	69.32
Control		90.00	0.00
S.E.±			0.42
C.D. at 1%			1.23

Current findings are also in agreement with Kushwaha and Verma (2014) [8], Shete *et al*. (2019) [17] were studied *in vitro*% inhibition of *F. oxysporum* and reported that *F. oxysporum* showed maximum sensitivity to *Trichoderma* spp. mutants than their wild type.

Efficacy of *Trichoderma* spp. against *Sclerotium rolfsii* at 7 DAI

Observations on per cent growth inhibition were recorded 7 DAI. The maximum% inhibition of *Sclerotium rolfsii* was recorded in *Trichoderma* spp. T_1 (*T. asperellum*) 67.07% which was followed by T_3 (*T. harzianum*) 66.42% then T_2 (*T. reesei*) 64.02% and T_4 (*T. hamatum*) 62.10%.

Plate 2: Efficacy of four *Trichoderma* spp. against *Sclerotium rolfsii* at 7 days after incubation

Table 3: Efficacy of *Trichoderma* spp. against *Sclerotium rolfsii* at 7 DAI

Tr. No.	*Trichoderma* spp.	(Mean) Radial Growth (mm)	Percent Growth Inhibition (%)
T_1	*T. asperellum*	29.63	67.07
T_2	*T. reesei*	32.38	64.02
T_3	*T. harzianum*	30.22	66.42
T_4	*T. hamatum*	34.11	62.10
Control		90.00	0.00
S.E.±			0.36
C.D. at 1%			1.23

Study are in aggrement with Vyawahare *et al*. (2019) [20], Shete *et al*. (2019) [17] which show maximum% growth inhibition against *Sclerotium rolfsii.*

Antagonistic activity of *Trichoderma* spp. against *Rhizoctonia bataticola* at 7 DAI

Observations on percent growth inhibition were recorded at 7 DAI (Day After Inoculation) by dual culture method. Maximum% inhibition was recorded in *Trichoderma* spp. T_1 (*T. asperellum*) 69.84% against *Rhizoctonia bataticola* which was followed by T_2 (*T. reesei*) 65.30% then T_3 (*T. harzianum*) 63.92%. Whereas the minimum% growth inhibition of *Rhizoctonia bataticola* was recorded in T_4 (*T. hamatum*) 56.83%.

Plate 3: Efficacy of four *Trichoderma* spp. against *Rhizoctonia bataticola* at 7 days after incubation

Table 4: Antagonistic activity of *Trichoderma* spp. against *Rhizoctonia bataticola* at 7 DAI

Tr. No.	*Trichoderma* spp.	Mean	% Inhibition
T_1	*T. asperellum*	27.14	69.84
T_2	*T. reesei*	31.23	65.30
T_3	*T. harzianum*	32.47	63.92
T_4	*T. hamatum*	38.85	56.83
Control		90.00	00.00
S.E.±			0.31
C.D. at 1%			0.99

Similar findings were recorded by Totawar and Somani (1999) [19], Prashanthi and Vaishnav (2000) [14] and Manandhar *et al*. (2019) [13] they revealed maximum% inhibition of *R. bataticola* with *Trichoderma* spp. *in vitro*.

References

1. Agrawal T, Kotasthane AS. Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. Springer Plus. 2012;1:73.
2. Bastakoti S, Belbase S, Manandhar S, Arjyal C. *Trichoderma* species as Biocontrol Agent against Soil Borne Fungal Pathogens Srijana. Nepal Journal of Biotechnology. 2017;5(1):39-45.
3. Benitez T, Carmen M, Limon Antonio C, Rincon M. Bio control mechanisms of *Trichoderma* strains. Int. Microbiology. 2004;7:249-260.
4. Bhat G, Bhat S, Kuruvinashetti MS. Screening of *Trichoderma* isolates for chitinolytic activity using glycol chitin plate assay. Asian Journal of Bio Sci. 2009;4(1):29-32.
5. Boregowda N, Puttaswamy H, Harohalli NS, Harishchandra SP, Nagaraja G. Total crude protein extract of *Trichoderma* spp. includes systematic resistance in pearl millet against the downy mildew pathogen. Biotech. 2017;3:183.
6. Giridhar D, Sankar R, Kumar NK, Rajanikanth D, Devamma N. Purification, Characterization and antifungal activity of chitinase from *Trichoderma viride*. J. of Cell and Tissue Res. 2012;12(2):3187-3192.
7. Jager G, Velvis H, Lamers JG, Mulder A, Roosjen J. Control of *Rhizoctonia solani* in potato by biological, chemical and integrated measures. Potato- Res. 1991;34:269-284.
8. Kushwaha M, Verma AK. Antagonistic activity of *Trichoderma* spp. against isolated and identified plant pathogens. 2014;IJCBS(1):2349-2724.
9. Kulkarni S, Ramanujan RJ, Rabinrdra M, Roa NR. Isolation, identification and documentation of efficient chitinase enzyme production ability strains of *Trichoderma*. J Pl. Dis. Sci. 2010;5(1)198-202.
10. Lowry OH, Bessey OA. Protein Measurement with the Folin Phenol Reagent. Biol. Chem. 1951;193:265-27.
11. Lugtenberg BJ, Dekkers L, Bloemberg GV. Molecular determinants of rhizoshhere colonization by *Pseudomonas*. Ann. Rev. Phytopathol. 2001;39:461-490.
12. Mahajan K, Dhange A, Sharma JK. Evaluation of *Trichoderma* spp. against *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* under *in vitro* condition. Int. J Microbial App. Sci. 2018;7:595-599.
13. Manandhar S, Pant B, Manandhar C, Baidya S. In- vitro evaluation of biocontrol agents against soil borne. Plant Pathogens Journal of Nepal Agriculture Reasearch Council. 2019;5:68-72.
14. Prashanti AM, Vaishnav MV. Biocontrol of *Rhizoctonia bataticola*. Indian Phytopathology. 2000;39(1):439.
15. Roberts WK, Selitrennikoff CP. Plant and Bacteria Chitinase Differ in Antifungal Activity. J General Microbiology. 1988;34:169-176.
16. Schipper B, Bakker AW, Baker PA. Interaction of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Ann. Rev Phytopathol. 1987;25:339-58.
17. Shete RS, Ingle ST, Totawar HM, Ghate AG. Enhanced chitinase production ability and morphological variation of *Trichoderma asperellum* mutants. Int. J Chem. Stu. 2019;7(5):183-188.
18. Schirmbock M, Lorito M, Wang YL. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl. Environ. Microbiol. 1994;60:4364-70.
19. Totawar MV, Somani RB. Some studies on bio-control of cotton root rotting fungus using *Trichoderma*. Abstr. State level seminar on plant disea vse management. Sailu Parbhani. 1999;4:25-26.
20. Vyawahare MP, Ingle ST, Gathe AG. Morphological variation and chitinase production ability of *Trichoderma viride* mutants. Int. J Curr. Microbial. App. Sci. 2019;8(01):447-455.