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Extent of lysis of *Rhizoctonia solani* Kühn cell wall preparation by different hyperparasites

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Purified cell wall of *Rhizoctonia solani* was treated with enzyme preparation of wellknown hyperparasites of *Rhizoctonia solani* viz *Trichoderma harzianum, T. viride, T. hamatum* and *Aspergillus flavus*. The extent of lysis of the cell wall of *Rhizoctonia solani* due to the enzymes of hyperparasites was studied with the help of nephelometer. *T. harzianum* caused maximum lysis of 86% by 25 h followed by *T. viride* (45%), *T. hamatum (15%) and A. flavus (6%)*. The optimum temperature for enzymic lysis of *Rhizoctonia solani* cell wall was found to be 20°C. The results indicate that *T. harzianum* can be used as biological control agent of *Rhizoctonia solani* in Chotanagpur plateau.

Key words : *Rhizoctonia solani,* lysis, hyperparasites, biological control agent

INTRODUCTION

Black surf, incited by *Rhizoctonia solani* Kühn, is a major disease of potato in the tribal region of Chotanagpur. The chemical measures to control the disease is not only costly but also less effective (Elad *et. al.*, 1983). Thus another approach which could be used to control the disease is the biocontrol agents particularly *Trichoderma* spp. (Papavizas, 1985). Mycoparasitism is considered to be an important mechanism of biological control and probably depends on the production of lytic enzymes (Chet, 1987). This article reports the comparative abilities of *Trichoderma* spp. and *Aspergillus flavus* to cause lysis of cell walls of *Rhizoctonia solani*.

MATERIALS AND METHODS

Culture

Rhizoctonia solani Kühn (No. 2755) isolated from potato tubers were obtained from IARI, New Delhi. Following hyperparasites of *Rhizoctonia solani* were obtained from IARI, New Delhi : 1) *Trichoderma viride* (Pers) Gray (No. K-133), 2) *Trichoderma harzianum* Rifai (No. 1894), 3) *Trichoderma hamatum* (Bon) Bain (No. 2084), 4) *Aspergillus flavus* Link-ex. Fries (No. 315)

Preparation of Rhizoctonia solani *cell wall*

Rhizoctonia solani growing on PDA slants was harvested after 20 days of growth in 5 ml of distilled sterile water from each slant. An aliquot of 25 ml of *Rhizoctonia solani* suspension was obtained from 5 slants. They were crushed and homogenesized in a homogenizer (A. HT Philadelphia, U.S.A). It was washed by repeated centrifugation (3000 rpm) with O.1m NaCI, O.M acetate buffer (pH=5.5) and distilled water until the cells were free from cytoplasmic materials. The wall was inactivated by heating at 100°C for 30 minutes in a boiling water bath. An aliquot 0.2, µM of Na_2NO_3 was then added to keep the preparation sterile.

Preparation of enzyme

Trichoderma viride, Trichoderma hamatum, Trichoderma harzianum, and Aspergillus flavus were grown for 7 days at 25°C in stationary cultures in 250 ml conical flasks containing 100 ml of mineral medium of following composition (g/l) at pH = 5.5 : Glucose (anhydrous), 10; Ammonium tartrate, 2; K_2HPO_4, 1; $MgSO_4$, $7H_2O$, 0.5 ; Leaf extract, 1; and Trace solution, 1 ml.

The trace element solution contained the following (in mg/l) : $Na_2B_4O_7$, $10H_2O$, 100 ; $ZnSO_4$, $7H_2O$,

70; $FeSO_4$, $7H_2O$, 50; $CuSO_4$, $5H_2O$, 10; $MnSO_4$, $4H_2O$, 10 ; $(NH_4)_6$ Mo_7O_{24}, $4H_2O$, 10.

The culture filtrate was separated from the mycelium by filtration through Whatman Filter No. 3. The filtrate was centrifuged at 3000 rpm for 30 min. The culture filtrate was then dialysed twice against two changes of distilled water for 48 h at 4°C. This resultant preparation was taken as that of enzyme for studies on lysis of cell wall of *Rhizoctonia solani.*

Measurement of Lysis

One mg of *Rhizoctonia solani* cell wall preparation was taken in 1 ml of 0.05 M borate-citrate-phosphate buffer (pH=5.5). This was incubated for different time (1 h, 5 h, 10 h, 15 h, 20 h and 25 h and temperature (5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C) with 1 ml enzyme preparation. Turbidity was measured in Nephelometric turbidity units (NTU) by Nephelometric method with the help of a Nephelometer (Systronic, India). The sample was shaken thoroughly and air bubbles were allowed to escape. Sample was diluted with one or more volumes of turbidity free water until turbidity level fell down within limits of 30 to 40 NTU. Turbidity in original sample was calculated from the turbidity of diluted sample and the dilution factor was known. The sample was, then, transferred to the turbidometer tube and direct reading was taken on the scale. The calcution was made as following.

Nephelometric Turbidity Units = A × (B+C)/C

Where A = NTU found in diluted sample, B=volume of dilution water in ml, C=sample volume taken for dilution in ml.

The percentage loss in turbidity was taken as measure of lysis of *Rhizoctonia solani* cell wall by enzymes of different hyperparasites. The boiled enzyme (30 min) was taken as control for the experiment.

RESULTS AND DISCUSSION

The degree of lysis of the cell wall of *Rhizoctonia solani* by enzymes of different hyperparasites is shown in Table 1. The enzymes of *Trichoderma harzianum* rapidly digested the cell wall of *Rhizoctonia solani*. It lysed 86% of the cell-wall by 25 h. *T.viride,* another hyperparasite, lysed only 45% of the cell wall in the same period whereas lysis brought about by *T. hamatum* and *Aspergillus flavus* was insignificant.

Table 1 : Degree of lysis (%) of *Rhizoctonia solani* cell wall preparation harvested after 20 days of growth on PDA slant at 30°C

Hyperparasites	Time (h)					
	1	5	10	15	20	25
Trichoderma viride	10	17	25	40	42	45
Trichoderma hamatum	8	13	13	13	15	15
Trichoderma harzianum	16	29	40	63	75	86
Aspergillus flavus	3	3	5	5	6	6

Results are mean of 3 replicates.

Bartnicki-Garcia (1973) who studied the cell wall chemistry of *R.solani* reported that cell walls mostly consist of glucans and 6-8% chitin. On the basis of this Hadar *et al.* (1979) suggested the role of lytic enzymes in degradation of *R. solani.* Our studies directly confirms the lysis of *R. solani* cell wall by enzymes of *T.harzianum* and *T.viride* as the inactivated wall preparation of *R. solani* was lysed with active enzyme preparation of the hyperparasites. This mechanism of hyperparasitization by *Trichoderma* is different from that reported by Sawant and Mukhopadhyay (1990) where its volatile and non-volatile toxic metabolites played major role in controlling the damping off disease of sugarbeet.

Hadar *et al.* (1979) used *T. harzianum* to control damping off of bean and tomato plant seedlings caused by *Rhizoctonia solani* and *Pythium aphanidermatum* respectively. The strain of *T. harzianum* used by them extracellularly expleted β-1,3 glucanase and chitinase (Chet and Henis, 1969). Elad *et al.* (1983), too, reported lysed sites and penetration holes at the area of contact of *R. solani* hyphae parasitized by *Trichoderma spp.* They also detected,, with scanning electron microscope and fluorescence microscope, high amount of expletion of β-1, 3 glucanase and chitinase in dual agar cultures where *T. harzianum* parasitized *Sclerotium rolfsiji.* Relatively low levels of β-1, 3 glucanase and chitinase were secreted with either fungus alone. The enzymatic activity diminished when antagonism was prevented by cycloheximide. Similar mechanism of *T. harzianum* action on *Rhizoctonia solani* cell wall is indicated in our studies.

The light and pH are not the major factors affecting hyperparasitism in nature and especially in soil (Shigo and Hillis, 1973). These factors nevertheless can influence host-parasite relation *in-vitro*. In view of this, the studies with light and pH were not conducted here. The experiments with temperature revealed that optimum temperature for degradation of inactivated cell wall of *R. solani* by enzymes of *T. harzianum* was 20^{0}C (Table 2). This indicates that the black scurf disease can effectively be controlled in field by adjusting the time of sowing of potato, when soil temperature is around 20^{0}C.

Table 2 : Degree of lysis of *Rhizoctonia solani* cell wall by enzyme preparation of *Trichoderma harzianum* at different temperatures

Incubation Temperature (^{0}C)	Lysis (%) of *Rhizoctonia solani* cell wall measured after 1 h of incubation
5	2
10	9
15	12
20	25
25	20
30	16
35	14

Results are mean of 3 replicates.

The potatoes which are normally sown in September-October (temperature around 25-30°C) give visible symptoms of *Rhizoctonia solani* infection in late November to early December when temperature ranges from 16-20°C. The experiments with temperature indicate that potato sowing may be delayed by a month (late Novermber to early December) when the temperature is around 20°C. This will reduce the infection of *R.solani* as by delayed sowing its superinfection by *T. harzianum* can be encouraged at the initial stage of the growth of potato.

The present study, thus, reveals that *T. harzianum* controls the infection of *R. solani* by effectively lysing its cell wall through its enzymes. The delayed sowing of potato, when the temperature is around 20°C, encourages the superinfection of *R.solani* by *T. harzianum*. This indicates that *T. harzianum* can be used as biological control agent to curb *R. solani* infection on potato in Chotanagpur plateau.

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