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Biological control of *Macrophomina phaseolina* by some stable mutants of *Trichoderma harzianum*

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Among the two native (Th_3 and Th_4) and six mutant isolates (50 Th_3II, 50 Th_3VI, 100 Th_3I, 150 Th_3I, 75 Th_4I and 125 Th_4I) of *Trichoderma harzianum* evaluated against *Macrophomina phaseolina* inciting stem rot of jute, 75 Th_4I was most effective than others with respect to inhibition of mycelial growth of the pathogen in dual culture technique. However, the 50 Th_3VI isolate caused the 71.48% inhibition of mycelial growth of pathogen followed by 150 Th_3I and 75 Th_4I through production of non-volatile antibiotics. In the pot experiments, higher germination and lowest seedling mortality were recorded with the application of 75 Th_4I mutants.

Key Words : Biocontrol, jute, *Macrophomina phaseolina*, mutant isolate, stem rot, *Trichoderma harzianum*

INTRODUCTION

Biological control is the reduction in inoculum density or disease producting activities of a pathogen or a parasite in its active or dormant state, by one or more organisms accompanied naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists (Cook and Baker, 1983). *Trichoderma* and *Gliocladium* spp., typical soil inhabitants, are well known for their antagonistic behaviour against many soil borne plant pathogens viz., *Rhizoctonia solani* (Howell, 1987 ; Elad *et al.*, 1981) *Sclerotinia sclerotiorum* (Tu, 1980), *Pythium ultimum* (Howell and Stipanovic, 1983 ; Lewis *et al.*, 1996), *Sclerotium rolfsii* (Henis *et al.*, 1983 ; Henis and Papavizas, 1983) and *Macrophomina phaseolina* (Pan *et al.*, 2001 ; Saha and Pan, 1996) etc. Biocontrol of plant pathogens seeks a solution in terms of restoring and maintaining the biological balance within the ecosystem and offers a powerful means to improve the health and hence the productivity of plant is increased by suppression or destruction of pathogens inoculum, protection of plants against infection or increasing the ability of plants to resist pathogens (Upadhyay *et al.*, 1996).

Biocontrol potential of *Trichoderma* spp., like fungicidal tolerance, survival ability in soil, antagonistic potentiality and ability to colonize plant parts, have been enhanced by mutation (Mukherjee and Mukhopadhyay, 1993 ; Troutman and Matejka, 1978 ; Selvakumar *et al.*, 2000). Ultra violet radiation induced benomyl tolerant mutants of *Trichoderma* spp. are more effective than wild type strain in controlling *R. solani, P. ultimum, S. rolfsii* etc. (Abd-El Moity *et al.*, 1982 ; Papavizas, 1982) but no more against *M. phaseolina.* Improved yield and enhanced cellulase activity by *T. viride* are also obtained by mutation and selection. (Montenecourt and Eveleigh, 1977). The present research work has been conducted to compare the biocontrol potential of some γ–radiated mutants of *T. harzianum* along with their native isolates against *M. phaseolina* induced stem rot of jute both *in vitro* and *in vivo.*

MATERIALS AND METHODS

Isolation of *T. harzianum*

Two different isolates of *T. harzianum* were isolated from rhizosphere soil of jute by dilution plate

technique (Waksman and Fred, 1922) on *Trichoderma* specific medium (Elad and Chet, 1983) modified by Saha and Pan (1997). The isolates were maintained on potato dextrose agar (PDA) slant at 4°C for subsequent use.

Isolation of pathogen

Virulent culture of *M. phaseolina* was isolated from infected jute stem by tissue segment method (Rangaswami, 1958) on potato dextrose agar medium. The culture was purified by hyphal tip culture method (Rangaswami, 1958).

Production of mutant isolates

Four-day-old culture of the two native isolates (Th_3 and Th_4) of *T. harzianum* grown on potato dextrose agar slant were exposed to non particulate electromagnetic γ ratiation with five different dosage of 50 KR, 75 KR, 100 KR, 125 KR and 150 KR to get mutant isolates (Roy and Pan, 1998). The phenotypically different colonies in respect of colour, texture and growth pattern over native isolate were selected. The selected isolates of *T. harzianum* were subcultured for ten generation of PDA plate. The mutant isolates that maintained the original phenotypic characters and hyperparasitic potentiality were considered as stable mutant and were used in the subsequent experiments.

Dual culture technique

Eight isolates of *T. harzianum* were screened for their antagonistic activity in dual culture technique (Morton and Stroube, 1955) against *M. phaseolina* on PDA in petriplates. Both the antagonist and the pathogen were inoculated at the same time. Disc of 5 mm cut from the margin of young vigorously growing cultures were placed at the opposite points in the plates 4 cm apart from each other. The petriplates were incubated at 28 ± 1°C. The antagonistic activity of each isolate was measured by using Bell' scale (Bell *et al.,* 1982). Types of interaction were studied for the seven days. Hyphal interactions were studied by growing them on cellophane membrane placed over solidified PDA in petriplates. After both the fungi came in contact with each other. The contact zone was cut using a sharp blade and taken out along the cellophone. It was washed gently with water, mounted under lactophenol-cotton blue over a clean glass slide observations were made under microscope.

Effect of non-volatile antibiotics

The method employed by Dennis and Webster (1971a) was adopted. Two native and six mutant isolates were cultured in 100 ml sterile potato dextrose broth in 250 ml conical flask with intermittent shaking. After ten days the culture filtrate was passed through Whatman No. 42 filter paper and collected in a sterile flask. The culture filtrate was sterilized by passing it through Millipore membrane fitter (0.4 µm pore size). 5 ml culture filtrate was added to 95 ml molten PDA medium. The medium was poured into petriplates and inoculated with 5 mm disc of *M. phaseolina* and incubated at 28±1°C for 4 days. Control plates were maintained without culture filtrate. Radial mycelial growth was recorded and per cent inhibition was calculated.

***In vivo* efficacy of native and mutant isolates of *T. harzianum* against *M. phaseolina* induced stem rot of jute**

The study was conducted in sterilized potting mixture containing loam soil and FYM @ 2 : 1 v/v. Sterilization was done by using 5% formaldehyde @ 1 l/c.f.t. and keeping potting mixture covered with polythene sheet for 7 days. *M. phaseolina* was mass multiplied in maize meal sand water (1 : 2 : 1 w/w) medium for 21 days. This inoculum was used to inoculate the pot @ 2.5 g/kg of potting mixture. The biocontrol agents were mass multiplied in rice husk containing around 50% moisture and c.f.u./g of mass multiplied antagonist was estimated on modified TSM. In 2 kg potting mixture required amount of mass multiplied antagonist was added to get a population of 10^5 c.f.u./g of potting mixture. In each pot, 5 g of compound fertilizer 10-26-26 (N-P-K) was added before sowing. Ten jute seeds (var. JRC-312) were sown in a pot at a equidistant position and irrigated with tap water. During entire period of experiments, measured quantity of water (500 ml/pot after every two days) was applied for irrigation and to maintain the moisture content

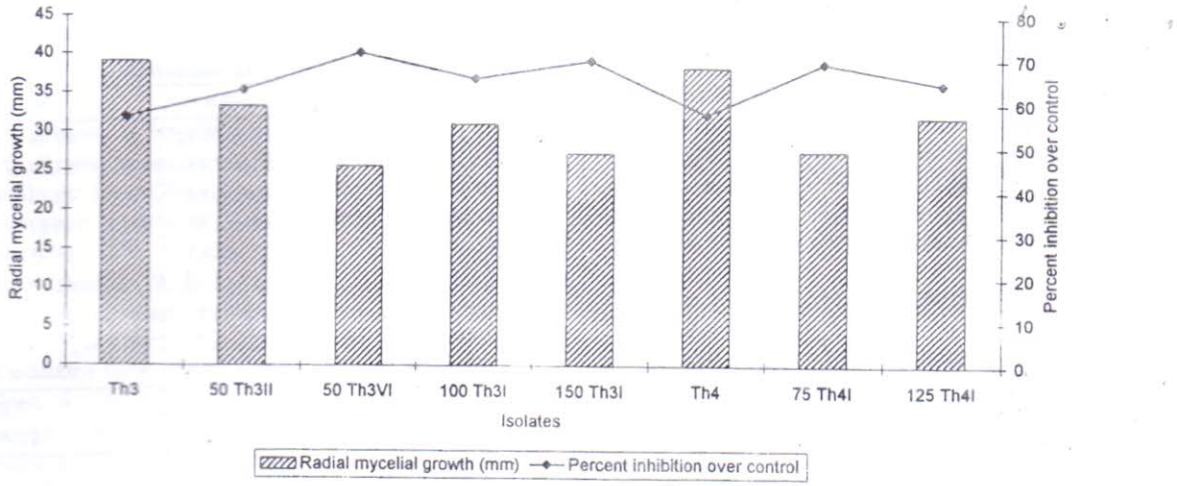

Fig. 1 : Effect on non-volatile antibiotic(s) of *T. harzianum* on growth of *M. phaseolina*

aroung 50%. The measurement of disease was scored as per cent plant mortality at two weeks interval.

RESULTS AND DISCUSSION

Interaction of isolates in dual culture

A reduction in the growth of the *M. phaseolina* was noticed when it was paired with antagonists 4 cm apart on PDA. Within the 6 days of inoculation majority of the isolates overgrew the pathogen colony. Among the two native (Th_3 and Th_4) and six mutant isolates of 75 Th_4I was most effective than others with respect to inhibition of mycelial growth of the pathogen. The microscopic examination at the point of contact of the pathogen and the antagonist revealed hyphal coiling, formation or penetration peg, leakage of cell wall followed by degradation of cell wall in the test pathogen.

Effect of non-volatile substances

All the isolates reduced the mycelial growth of the pathogen through the production of non-volatile antibiotics (Fig. 1). 50 Th_3VI isolate caused the 71.48% inhibition of the mycelial growth of the pathogen followed by 150 Th_3I and 75 Th_4I. The native isolate Th_3 and Th_4 produced very low non-volatile antibiotics than others isolates in culture filtrate.

In vivo efficacy against pathogen

There was a direct effect of the antagonist isolate on germination of jute seed, in pot (Table 2) except the Th_4 isolate. In case of *in vivo* biocontrol potential of native and mutant isolate of *T. harzianum* it was observed that all the isolate reduced the per cent plant mortality significantly. As the disease progress was very slow, variation in the effect of the isolates could be determined only after 28 DAS. The mutant isolate 75 Th_4I showed significantly higher efficiency causing only 21.11% seedling mortality at 56 DAS.

Table 1 : Screening of antagonistic potential of isolates of *T. harzianum* against *M. phaseolina* by dual culture technique.

Isolate	Duration requires for point of contact (days)	Bell's scale after (days)*		
		3	4	5
Th_3	2	R_3	R_2-R_1	R_1
50Th_3II	2	R_3	R_2	R_1
50Th_3VI	2	R_3-R_2	R_1	
100Th_3I	2	R_2-R_1	R_1	
150Th_3I	2	R_3	R_1	
Th_4	2	R_4-R_3	R_2	R_1
75Th_4I	2	R_1		
125Th_4I	2	R_3-R_2	R_1	

Trichoderma represented the most widely and thoroughly studied fungus exhibiting antagonistic

Table 2 : *In vivo* efficacy of wild and mutant isolates of *T. harzianum* against *M. phaseolina.*

Isolate	Germination (%)	Per cent plant mortality induced by *M. phaseolina*			
		14 DAS	28 DAS	42 DAS	56 DAS
Th_3	68.89 ± 14.99	0.00	34.44 ± 12.77	46.11 ± 10.92	49.44 ± 15.30
$50Th_3II$	80.00 ± 8.16	0.00	4.17 ± 1.01	45.83 ± 7.43	53.24 ± 12.02
$50Th_3VI$	81.11 ± 6.85	0.00	12.29 ± 3.21	32.40 ± 8.59	48.74 ± 4.26
$100Th_3I$	75.55 ± 3.14	0.00	11.87 ± 4.47	23.74 ± 8.95	40.62 ± 4.30
$150Th_3I$	64.44 ± 17.50	5.81 ± 4.12	14.14 ± 7.68	32.32 ± 8.63	35.35 ± 7.99
Th_4	50.00 ± 8.16	0.00	8.33 ± 1.36	39.44 ± 7.50	42.78 ± 12.20
$75Th_4I$	85.00 ± 10.80	0.00	9.44 ± 8.20	9.44 ± 8.20	21.11 ± 13.96
$125Th_4I$	67.78 ± 20.79	0.00	9.76 ± 7.48	18.10 ± 5.67	31.56 ± 4.80
Control	60.00 ± 21.60	0.00	74.08 ± 18.89	88.89 ± 15.71	93.33 ± 9.43
S.Em ±	9.82	0.97	6.34	6.69	7.21
CD (P = 0.05)	29.17	2.88	18.82	19.87	21.40

activity against a range of soil borne plant pathogens (Howell, 1987 ; Lewis *et. al.,* 1996 ; Sivan and Chet, 1986 ; Upadhyay and Mukhopadhyay, 1983). Several possibilities of existence of fungal interaction have been evidenced, of which formation of appressoria, hook shaped contact branches and coiling around the host cell are most common (Elad *et al.,* 1983). Variability in antagonistic potentiality among the different isolates of *Trichoderma* against different pathogens has been reported (Highley and Ricard, 1988 ; Saha and Pan, 1996). Denis and Webster (1971b) reported the coiling of isolates of *Trichoderma* can contact with pathogen as well as delayed coiling with other isolates and no interaction with some other isolates of *Trichoderma.* Pathogen could not be reisolated from the zone of interaction revealing the total lysis of the pathogen. *Trichoderma* spp. antagonistic to range of fungi is reported to produce different types of non-volatile antibiotics (Upadyhay and Mukhopadhyay, 1983).

Mutant isolates showed much more antagonistic activity against pathogen both *in vitro* and *in vivo.* The probable reason is that mutation by γ-radiation alter the genetic make up of the native isolate with enhancement of antibiotic and enzyme production *in vitro* and more competitive saprophytic ability and tolerance to adverse effect in soil. Many authors have reported that mutation increase the antagonistic activity of the biocontrol agents (Mukherjee and Mukhopadhyay, 1993 ; Selvakumar *et al.,* 2000).

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