J. Biol. Control, 11: 43-47, 1997

Inhibition of *Macrophomina phaseolina* (Tassi.) Goid by mutants of *Trichoderma viride* Pers. ex Fr.

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ABSTRACT: Nine mutants of *Trichoderma viride* Pers. ex Fr. obtained by using different mutagenic agents were evaluated for their relative efficacy against *Macrophomina phaseolina* (Tassi.) Goid in terms of antibiotic production. The cell free culture filtrate of mutant M1 showed the highest *in vitro* inhibition of mycelial growth (54.4%) and sclerotial germination (75.8%) of *M. phaseolina* followed by M3 and they were on par with each other. The mutants M3 and M8 produced maximum volatiles *in vitro* as evidenced by the retardation in growth of *M. phaseolina* which registered a growth of 10 mm after 48 h of incubation while in control the growth was 90 mm.

KEY WORDS: Antibiosis, *Macrophomina phaseolina*, non-volatiles, *Trichoderma viride* mutants, volatiles

Antibiosis plays an active role in the biocontrol of plant diseases and often acts in concert with competition and or parasitism. Dennis and Webster (1971 a, b) described the antagonistic properties of *Trichoderma* in terms of antibiotic production. 'Trichodermin', a sesquiterpene antibiotic of *Trichoderma* spp. was found to be active against fungi (Wright, 1956). *Trichoderma* spp. also release volatiles which produce fungistatic effects (Dick and Hutchinson, 1966). Cell free culture filtrates or extracts of these filtrates demonstrate the role of antibiosis as reported by Tamimi and Hutchinson (1975) who found that volatile component of *Trichoderma* spp. caused inhibition of mycelial growth of *M. phaseolina*. The mutants of *T. viride* were tested in this study for their relative efficacy against *Macrophomina phaseolina* in terms of antibiotic production.

Part of Ph. D. thesis submitted by the first author to Tamil Nadu Agricultural University, Coimbatore 641 003
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MATERIALS AND METHODS

Nine mutants (M1 to M9) of *T. viride* were obtained using mutagenic agents *viz.*, ultra-violet irradiation, gamma irradiation and UV + ethyl methane sulphate and tested for their inhibition efficacy on *M. phaseolina in vitro.*

The toxic metabolites produced by the mutants of *T. viride* were tested following the method of Lifshitz *et al.* (1986) with a little modification. The wild-type and its mutants were grown in 50 ml of potato dextrose broth (PDB) taken in 250 ml conical flasks. The cultures were incubated for 7 days at room temperature (28 ± 2°C). Uninoculated broth served as control. After incubation the mycelial mats were separated and the growth medium was centrifuged at 3000 rpm to concentrate the conidia. The clear supernatant solution was taken and filtered through Whatman No. 42 filter paper. Three ml of the cell free culture filtrate of each strain was mixed with 12 ml of potato dextrose agar (PDA) medium taken in petri dishes. An 8 mm mycelial disc of *M. phaseolina* was inoculated at the centre of each petri dish and incubated at room temperature (28 ± 2°C). The linear growth of the colony in each petri dish was measured. Sclerotia were taken after 24 and 48 h. From each petri dish, 25 matured sclerotia were taken and their germination was tested by slide germination method. They were incubated in moist chamber for 24 h. The number of germinated sclerotia was counted using a microscope.

The influence of volatiles produced by *T. viride* and its mutants on the growth of *M. phaseolina* was estimated utilising the method described by Bruce *et al.* (1984). Agar disc (8 mm diam.) from the actively growing margins of *T. viride* and its mutants were aseptically removed and placed in the centre of petri dishes containing 3 per cent malt extract agar medium. Mycelial discs removed from the actively growing culture of *M. phaseolina* were inoculated in a similar manner to petri dishes and then inverted over the plates inoculated with antagonists. A thin polythene membrane was placed between the two dishes and sealed with adhesive tape. Control plate consisted of *M. phaseolina* inverted over uninoculated malt extract agar medium. The plates were incubated at room temperature (28 ± 2°C) and three replicates were maintained for each treatment. The linear growth of *M. phaseolina* was measured after 24 and 48 h. Discs of *M. phaseolina* inhibited by volatiles of *T. viride* and its mutants were transferred on to PDA medium in order to test the fungicidal / fungistatic effect.

RESULTS AND DISCUSSION

The culture filtrates of all mutants significantly reduced the mycelial growth of *M. phaseolina*. Dennis and Webster (1971 a, b) observed wide variation between isolates of same species as well as different species of *Trichoderma* in antibiotic production. In the present study, culture filtrate of mutant M1 showed the highest inhibition of mycelial growth after 24 h (70.5%) and 48 h (54.4%) and sclerotial germination (75.8%) of *M.*

phaseolina. However, M3 was on par with M1 (Table 1). The non-volatile antibiotics produced by the mutants M1, M3, M7 and M2 inhibited *M. phaseolina* more efficiently than the parent isolate. Similar observations were made by Dennis and Webster (1971 a) and Pande (1985).

Table 1. Inhibition of *M. phaseolina* by culture filtrates of *T. viride* mutants

Mutant	Mycelial growth (mm) after 24 h	Per cent inhibition of mycelial growth	Mycelial growth (mm) after 48 h	Per cent inhibition of mycelial growth	Sclerotial germination (%)	Per cent inhibition of sclerotial germination
P	24	55.6	53	41.1	33.7 (35.47)	65.3
M1	16	70.5	41	54.4	24.0 (29.32)	75.8
M2	20	62.9	49	45.6	28.0 (31.94)	71.2
M3	17	68.5	43	52.2	25.3 (30.22)	73.9
M4	31	42.5	68	24.4	49.0 (44.43)	49.1
M5	25	53.6	56	37.8	36.3 (37.07)	62.5
M6	30	44.4	65	27.8	46.7 (43.09)	51.9
M7	18	66.6	44	51.1	26.3 (30.87)	72.8
M8	24	55.6	55	38.9	34.0 (35.67)	64.9
M9	26	51.9	59	34.4	39.7 (39.03)	59.1
Control	54	-	90	-	97.0 (80.12)	
SEM ±	1.0		0.9		0.6	
CD (P=0.05)	2.9		2.8		1.8	

Figures in parentheses are arcsine transformed values

Results on inhibition of *M. phaseolina* due to volatile compounds of *T. viride* and its mutants indicated that all the mutants inhibited the mycelial growth of *M. phaseolina* after 24 h of incubation. After 48 h mutants M3 and M8 registered the highest inhibition of mycelial growth (88.9%) followed by M4 (46.7%) (Table 2). Other mutants did not show any difference since the growth of mycelium reached a maximum of 90 mm. Dennis and Webster (1971 b) found greater variation in the effects of the gases from cultures of different strains and species of *Trichoderma*. In the present study, when the mycelial discs from different treatments were transferred to PDA medium, grew normally, thereby suggesting that the volatiles had only fungistatic effect against *M. phaseolina*. Umamaheswari (1991) also observed the fungistatic effect of volatiles of *T. viride* against *M. phaseolina*. Among the mutants, M3 produced the higher amounts of both volatiles and non-volatiles than the wild-type strain as evidenced by the maximum retardation of growth of *M. phaseolina*.

Table 2. Effect of volatiles of *T. viride* mutants on *M. phaseolina*

Mutant	Linear mycelial growth (mm) of *M. phaseolina* after		Per cent inhibition of mycelial growth over control after	
	24 h	48 h	24 h	48 h
P	45.0	90.0	19.6	0.0
M1	44.0	90.0	21.4	0.0
M2	43.0	90.0	23.2	0.0
M3	10.0	10.0	82.1	88.9
M4	35.0	48.0	37.5	46.7
M5	48.0	90.0	14.3	0.0
M6	42.0	90.0	24.9	0.0
M7	44.0	90.0	21.4	0.0
M8	10.0	10.0	82.1	88.9
M9	42.0	90.0	25.0	0.0
Control	56.0	90.0		
SEM ±	0.9	0.5		
CD (P=0.05)	2.8	1.4		

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