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ELUCIDATION OF THE ANTAGONISTIC VARIATIONS AMONG ISOLATES OF *TRICHODERMA* sp. AGAINST *FUSARIUM OXYSPORUM*

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Variations in antagonistic activities of ten isolates of *Trichoderma* sp. were studied *in-vitro* against *Fusarium oxysporum*, the incitant of damping off and rots of conifers. All the isolates inhilbited mycelial growth of test fungus to varying degrees mainly through the production of diffusible substances with apparently no production of volatile metabolites. Few of the isolates showed stimulatory effect on mycelial growth.

Keywords : *Trichoderma* sp.; *Fusarium Oxysporum;* Biological Control.

Introduction

Several recent studies have demonstrated the importance of naturally occurring biological phenomena such as supressive soils, mycorrhiza, antagonistic microbes etc., augmenting the crop yields by supression or destruction of plant pathogens. Among the promising antagonists, *Trichoderma* sp. are the common fungi with a well demonstrated antagonistic properties against a wide range of soil borne fungi. *Fusarium oxysporum* Schlecht is a universal nursery pathogen causing daming off and root rots of sevaral forest plants. In this study ten isolates of *Trichoderma* sp. were evaluated for the potential antagonistic variations against *F. oxysporum* under *in-vitro* conditions.

Meterial and Methods

Ten isolates of *Trichoderma* sp. used in this study were isolated from the soils from different regions of France (Table 1) and maintained on P.D.A. slants. The pathogenic isolates of *Fusarium oxysporum* Schlecht was isolated from damped off seedlings of *Pseudotsuga menziesii* Mirb. France (Douglas-fir) and maintained on P.D.A.

Paired culture tests—Each isolate of *Trichoderma* was tested for the antagonistic potential in dual cultures

against *F. oxysporum* on malt agar in Petri Plates. Mycelial discs (4 mm dia.) taken from margin of six days old culture was placed at the opposite pheriphery of the Petriplate and incubated at 23°C with culture of *F. oxysporum* or *Trichoderma* sp. alone serving as control. Mycelial growth of both the cultures were measured at 24 hrs interval and once the mycelium overlapped each other, the hyphal interactions were studied by removing the contact zone intact and observation under microscope.

Test for production of non-volatile (diffusible) inhibitory substances— Technique described by Dennis and Webster (1971b) was employed wherein malt agar medium in petriplates was covered by a sterile cellophane film. After 24 hrs agar discs (4 mm dia.) cut from margin of 6 days old culture of each of ten isolates of *Trichoderma* sp. were inoculated in the centre and incubated with 5 replicates per isolate. After 48 hrs the cellophane film along with the *Trichoderma* colony was carefully removed allowing only the non-volatile substances to diffuse into the medium. Mycelial disc (4mm dia.) of *F. oxysproum* was placed in the centre of petri plate and incubated at 23°C. Colony diameter of *F. oxysporum* was measured at 24 hrs interval and compared to that of control and the growth inhibition expressed as percentage.

Test for production of volatile inhibitory substances— Each strain of *Trichoderma* was grown on malt agar in Petri plates for 6 days at 23°C. Later, the

Table 1. Origin of different isolates of *Trichoderma* sp.

Isolate	Species	Origin
TA-1	*Trichoderma aureoviridae*	Epoisses
TI-1	*T.* sp. (Indeterminate)	Dijon
TI-2	*T.* sp. (Indeterminate)	Montpellier
TI-3	*T* sp. (Indeterminate)	Bondeaux
TH-1	*T. harzianum* Pers.	Montpellier
TH-2	*T. harzianum* Pers.	Dijon
TH-3	*T. harzianum* Pers.	Paris
TH-4	*T. harzianum*	Bordeaux
TV-1	*T. viridae* Rifai	Paris
THH-1	*Trichoderma hamatum* Pers.	Montpellier

upper lid of each Petritplate was replaced by a bottom lid containing malt agar inoculated with inoculum disc of *F. oxysporum*. The two dishes were taped together with adhesive tapes. Lids of petriplates without inoculation with strains of *Trichoderma* sp. being replaced in the same manner served as controls. After 48 hrs of incubation, the colony diameter of test fungus was measured and compared to that of control. The percentage of growth inhibition in all the experiments was calculated.

Results and Discussion

Mycelial growth of *F. oxysporum* was inhibited to variable extent by different isolates of *Trichoderma* sp. in the paired culture tests (Table 2). Intense mycelial growth inhibition ranging from 50 to 60 per cent by sevaral isolates was obsesved during the first 24 hrs of incubation which decreased to 5 to 15 per cent after 48 hrs without any significant variations among the isolates. On the contrary, few isolates, such as TI-3, TH-2, TH-3, TH-4 and TV-1 had growth stimulation (7 to 15 per cent). At 72 hrs, isolate THH-I was significantly different from others in stimulating the growth (78 per cent) followed by TH-3 (16 per cent), while other strains showed inhibition ranging from 4 to 33 per cent. Several earlier reports have indicated variations in the degree of inhibition of several fungi by *Trichoderma* sp. (Dennis and Webster, 1971a, b; Hutchinson and Cowan, 1972, Papavizas, 1985). The fungal colonies of the antagonist and test fungus grew and developed contact with each other after 72 hrs, leading to interpenetration of mycelium. Microscopic observation of the zone of contact did not show any form of mycoparasitism such as coiling around or penetrating hyphae of *F. oxysproum*. This result is in conformity with the work of Dennis and Webster (1971c) who reported the coagulation and vacuolation of cytoplasm of the hyphae of *Fomes annosus* and *Rhizoctonia solani*, while no such mycoparasitism was observed with *Fusarium* sp. by the same isolate of *Trichoderma*.

Production of non-volatile antibiotics by different isolates when tested by agar layer technique showed considerable mycelial inhibition ranging from 30 to 100 per cent during the first 24 hours of confrontation (Table 2). Isolates TH-1, TH-2 and TH-3 were highly effective with 100 per cent of growth inhibition but without any siginficant variations among the isolates except isolate TV-1. At 48 hours of incubation, there was gradual decrease in inhibition percentage (2 to 88 per) cent) with two isolates TI-1, TH 4 showing even growth stimulation. Substantial variations in production of antibiotics was observed between

Table 2. Percent growth inhibition of *F. oxysporum* by Isolates of *Trichoderma*

Test	Incubation time in hrs.	TA-1	TI-1	TI-2	TI-3	TH-1	TH-2	TH-3	TH-4	TV-1	THH-1
Dual Culture Test	24	50.5a	48.3a	55.0a	48.3a	55.0a	52.8a	52.8a	50.5a	59.5a	55.0a
	48	15.3a	15.3a	13.8a	+10.7a	15.3a	+10.7a	+4.5a	+7.6a	+15.3a	4.6a
	72	20.6a	4.76a	14.2a	33.3a	30.1a	30.1a	+15.8a	20.6a	26.9a	+77.6b
Non-Volatile Inhihitors	24	94.0a	73.2a	41.6a	96.5a	100.0a	100.0a	100.0a	50.0a	31.6b	66.6a
	48	26.4a	+10.2b	26.4a	1.5b	41.1a	61.7a	88.2a	+5.8b	29.4a	29.4a
	72	8.3a	19.9b	6.6a	16.6a	16.6a	16.6a	28.3a	+19.9b	8.3a	+16.6b
Volatile Inhibitors	24	0.0a	20.0a	20.0a	12.0a	20.0a	12.0a	4.0a	4.0a	+12.0a	12 6a
	48	0.0a	+11.9a	3.9a	3.9a	+1.3a	19.9a	6.6a	0.0a	11.9a	9.3a
	72	12.5a	+ 5.0a	7.5a	+12.5a	7.5a	+ 5.0a	+5.0a	0.0a	8.0a	+12.5

the isolates of the same species as well as between isolates of different species group, which is in accordance with the earlier reports (Dennis and Webster, 1971a b; Papavizcs and Lumsden, 1980; Cook and Baker, 1983).

When tested for the production of volatile metabolites in Petriplates, there was marginal evidence of growth inhibition of *F. oxysporum* (0-20 per cent) during first 24 hrs of confrontation (Table 2), which further decreased with prolonged incubation period of 48 hours. Isolates TI-1, TI-3, TH-2, TH-3 and THH-1, were found to stimulate the mycelial growth of the fungus (5 to 13 per cent) at 72 hours of incubation without any variations among the isolates.

Wide variations in the per cent of mycelial inhibition of *F. oxysporum* was observed with all the isolates of *Trichoderma* sp. used in this study. Earlier results indicate that isolates of *Trichoderma* have varying degrees of antagonium *in-vitro* against several pathogens and frequently a particular isolate antagonistic against one pathogen was often ineffective against others in cultures (Dymovich, 1960; Wells and Bell, 1979; Papavizas and Lumsden, 1980; Cook and Baker, 1983; Elad *et al.*, 1983). The decreased growth inhibition with prolonged incubation was observed equally well with all the three methods of evaluation for majority of isolates. This shows that either the inhibitory substances are not stable or they are subjected to degradation by their chemical nature or due to the action of *F. oxysporum*. Much evidence has accumulated showing the variations in the fungal susceptibility to the metabolites elaborated by *Trichoderma* sp., with *Fusarium* sp. in general being least receptive (Dymorich, 1960; Dennis and Webster, 1971 a, b; Mirkova, 1983; Sivan and Chet, 1984). Growth stimulation of the test fungi was observed without few isolates and it is possible that *Trichoderma* isolates produced diffusible growth metabolites in different proportions with varying effect on test fungi or may produce more than one active metabolite (Papavizas, 1985). Results from these studies are not expected to be necessarily related to the degree of biological control that may be observed in the field but reflect the capacities and variability of isolates of *Trichoderma* as potential antagonists.

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