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Cultural variability in *Trichoderma* spp. isolated from North-Eastern Hills of Tripura and Mizoram

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The morphological and cultural characteristics of 45 isolates of *Trichoderma* spp. isolated from soil samples of 17 locations of Tripura and 13 locations of Mizoram were studied. No isolate of *Trichoderma* was obtained from 3 locations of Tripura and 5 locations of Mizoram. On the basis of Morphological and cultural characteristics, all the 45 isolates of *Trichoderma* spp. were grouped into 11 categories.

Key words : *Trichoderma* spp., isolates, Tripura, Mizoram

INTRODUCTION

Trichoderma Pers. ex Fr., a ubiquitous fungus distributed widely in soils besides colonizing the roots, seeds, litter and other substrates, has assumed great significance as a biocontrol agent against several plant pathogens (Mukhopadhyay and Mukherjee, 1996 ; Papavizas 1985). Already, several *Trichoderma* products with trade names like Ecofit, Niprot, Bioderma, Bhasderma, F. Stop, Trichodex, Binab-T, M.T.R.-35 and T-39, are available globally as biological control formulations (Nagamani *et al.*, 2002). Like many other microbes, a great and wide variabilities exist in the characteristics of species of *Trichoderma* and many specific and sub-specific taxa exist. Recently, Nagamani *et al.* (2002) in their comprehensive study, characterized 13 Indian *Trichoderma* species based on morphological and cultural characters.

Variability in *Trichoderma* spp. with regard to the cultural characters and antagonistic ability would depend on the ecological region of their evolution and such studies have been reported on requirements for sporulation of sub-tropical isolates of *Trichoderma* spp. (Sangle *et al.*, 2003). North Eastern Hill (NEH) region has not yet been fully explored for variability of *Trichoderma* spp. and therefore, an attempt has been made to study it in the soils of citrus orchards spread across the villages of Tripura and Mizoram States.

MATERIALS AND METHODS

For isolation of *Trichoderma* species, soil samples were collected in the month of May from citrus growing 30 locations (Government nurseries as well as farmers' orchards) of Tripura and Mizoram and the *Trichoderma* spp. were isolated from samples following dilution plate method (Sangle and Bambawale, 2004). Martin's rose-bengal agar medium (Martin, 1950) was used for isolation of *Trichoderma* spp. Inoculated plates were incubated at 25°C under 12 hrs alternate fluorescent light and darkness. The colonies (number) were counted after 5-7 days, purified and were confirmed as *Trichoderma* following Rifai (1969). The cultures were maintained on PDA slants.

To study the cultural variablity of *Trichoderma* spp., 5 mm mycelial discs of each isolate were inoculated aseptically in the center of 90 mm petri-plates (three replicates) containing 20 ml of PDA. Inoculated plates were incubated at 25°C and the

observations of various colony characters were made after 48 hrs of incubation and subsequently, at 24 hrs intervals up to 10 days. The radial growth of each isolate was also measured at an interval of 12 hrs after incubation up to 3 days. Observations on the growth of the different isolates of PDA were recorded on presence or absence of aerial mycelia i.e. fluffy or suppressed growth, sporulation (abundant : 4-8 × 10^9 spores/petriplate ; moderate : 1-4 × 10^9 spores/petriplate and poor : < 1 × 10^9 spores/petriplate), presence of ring and pigmentation after 7 days of incubation, and rate of growth (very fast : > 30 mm per day, fast : 27-30 mm per day, medium : 23-27 mm per day and slow : < 23 mm per day) following Domsch *et al.* (1980) and Cook and Baker (1983).

RESULTS AND DISCUSSION

A total of forty-five isolates of *Trichoderma* were obtained from soil samples collected from the Tripura and Mizoram States (Table 1). Out of the

Table 1 : Isolates of *Trichoderma* spp. ; their relative growth rates and category

Place of collection	Details of collection			Colony growth		Isolate category*
	Collection	Species Identified	Isolate No.	Growth Diam. (mm) (at 72 hrs.)	Rate	
Vangmun	3	*T. harzianum*	1a, b, c	90	Fast	VI
	1	*Trichoderma* sp.	1d	56	Slow	I
Behliang Chip	1	*T. viride*	2	84	Fast	X
Behliang Chip	2	*Trichoderma* spp.	4a, b	77	Medium	I
	1	*T. harzianum*	4c	90	Very Fast	IV
Behliang Chip	1	T. *harzianum*	5a	>90	Very Fast	XI
	2	*Gliocladium* (*Trichoderma*) spp.	5b, c	>90	Very Fast	VI
Tlaksih	1	*T. viride*	6a	85	Fast	VII
	2	*Trichoderma* spp.	6b, c	70	Medium	IX
Tlaksih	3	*T. harzianum*	7a, b, c	90	Fast	III
	1	*T. viride*	7d	75	Medium	VII
Bangla	2	*Trichoderma* spp.	8a, b	60	Slow	III
	1	*T. virens*	8c	>90	Very Fast	XI
Tlangsang	2	*T. hamatum*	9a, b	78	Medium	VII
Sabual	2	*T. hamatum*	10a, b	>90	Very Fast	V
Sabual	1	*T. hamatum*	11a	90	Fast	VII
	1	*Trichoderma* spp.	11b,	46	Slow	III
	1	*T. viride*	11c	90	Fast	VII
Hmunpui	1	*Trichoderma* sp.	13	>90	Very Fast	I
Monchuang	1	*T. viride*	14a	89	Fast	IX
	1	*Trichoderma* sp.	14b	66	Slow	IV
Hmunpui	1	*Trichoderma* sp.	15	90	Fast	X
Nabinchera	1	*T. harzianum*	16a	>90	Very Fast	VI
	2	*Trichoderma* spp.	16b, c	77	Medium	X
	1	*Gliocladium* (*Trichoderma*) sp.	16d	90	Fast	IV
Thindawn Kolasib	1	*Trichoderma* sp.	18	87	Fast	I
Bualpui Kolasib	1	*T. viride*	19	80	Medium	I
Melthum	1	*T. harzianum*	23	90	Fast	IX
Sateek	1	*Gliocladium* (*Trichoderma*) sp.	25	90	Fast	XI
Sateek	1	*T. harzianum*	26	90	Fast	III
Muallungthu	1	*Trichoderma* sp.	28	90	Fast	VIII
Tuirial	2	*T. viride*	29a, b	90	Fast	X

CD 5% 3.65

*Based on Table 2.

soil samples from 17 locations in Tripura State, 36 isolates of *Trichoderma* were isolated from 14 locations and none from the remaining three locations. These included 12 isolates of *T. harzianum*, 5 of *T. viride*, 3 of *Gliocladium* (*Trichoderma*) spp. and 1 each of *T. hamatum* and *T. virens*. In the soil samples from 13 locations of Mizoram State, 9 isolates of *Trichoderma* were isolated from 8 locations and none from the remaining 5 locations. In this case 5 isolates of *T. harzianum*, 3 of *T. viride*, 1 of *Gliocladium* sp. (*Trichoderma* sp.) were obtained. Thirteen isolates from Tripura and 3 isolates from Mizoram resembled the characteristics of *Trichoderma* genus but could not be placed in any of the *Trichoderma* species described by Rifai (1969).

Table 2 : Grouping of growth characters on PDA

	Characters	Group No.
1.)	Fluffy mycelial growth	
1.1	With poor sporulation	
1.1. i	One ring formation and pigmentation present	: I
1.1.ii	One ring formation and pigmentation absent	: II
1.1.iii	No ring formation and pigmentation present	: III
1.2	With moderate sporulation	
1.2.i	One ring formation and pigmentatin absent	: IV
1.2.ii	More than one ring formation and pigmentation absent	: V
2.)	Supressed mycelial growth	
2.1	With poor sporulation	
2.1. i	No ring formation and pigmentation present	: VIII
2.2	With moderate sporulation	
2.2.i	No ring formation and pigmentatin absent	: IX
2.2.ii	More than one ring formation and pigmentation present	: X
2.3.i	With abundant sporulation	
2.3.ii	More than one ring formation and pigmentation absent	: XI

Out of the 45 isolates of *Trichoderma* obtained from the two States, 33 were subjected to further characterization based on colony characters. Out of them, 6 isolates were very fast growing (Table 1) and covered the plates before 72 hrs of incubation. Majority of the isolates (24) showed the presence of aerial mycelium and only 9 isolates showed suppressed growth. With respect to the extent of sporulation, 15 isolates had abundant sporulation, 10 medium and 8 isolates had low sporulation. Majority of the isolates (18) showed ring at the centre, 11 showed ring at the margin whereas 4 isolates showed no ring. Further, 8 isolates produced bright yellow pigmentation, 10 isolates gave yellow pigmentation and 15 isolates produced no pigmentation on the substrate. On the basis of these four characters, 33 isolates were grouped into 11 distinct categories (Table 2). Only one isolate each belonged to the categories II, IV and XI. Majority of the isolates (6) belonged to category VI in which there was fluffy mycelial growth, abundant sporulation, more than one ring formation and presence of pigmentation. Out of the very fast growing isolates, pigmentation was absent in 5 isolates (Nos. 4c, 5a, 5b, c, 10a, b and 16a) and only one isolate (N. 13) had pigmentation. Thus, there appears to be inverse correlation between the rate of growth and pigmentation. In these very fast growing isolates, abundant sporulation was noticed in 3, moderate in 2 and poor sporulation in 1 isolate. Regarding growth rate of the other isolates, 4 were fast growing, 6 were medium and 3 were slow growing.

No attempt was made to relate cultural characteristics with the pathogenic or otherwise ability of the fungal species. The observations, however, confirmed the variability in culturalal growth of a species collected from different locations. Inverse correlation between the rate of growth and pigmentation on PDA plates was also indicated.

REFERENCES

Cook, R. J. and Baker, K. F. 1983. *The nature and practice of biological control of plant pathogens.* American Phytopathological Society, St. Paul, Minn. USA, 539 p.

Domsch, K. H., Gams, W. and Anderson, T. H. 1980. *Compendium of soil fungi.* Vol. I, Academic Press, New York, pp. 794-809.

Martin, J. P. 1950. Use of acid, rose-bengal and Streptomycin in the plate method for estimating fungi. *Soil Sci.* **69** : 215-232.

Mukhopadhyay, A. N. and Mukherjee, P. K. 1996. Fungi as Fungicides. *Inter Jour Trop. Pl. Dis.* **14**(1) : 1-17.

Nagmani, A., Manoharachary, C., Agarwal, D. K. and Chowdhury, P. N. 2002. *Monographic contribution on Trichoderma.* Associated Publ. Co., New Delhi, 47p.

Papavizas, E. C. 1985. *Trichoderma* and *Gliocladium* : biology, ecology and potential for biocontrol. *Ann. Rev. Phytopathol.* **23** : 23-54.

Rifai, M. A. 1969. A revision of the Genus *Trichoderma. Mycol. Papers* **116** : 1-56.

Samuels, E. J. 1996. *Trichoderma* : a review of biology and systematic of the genus. *Mycol. Res.* **100** : 923-935.

Sangle, U. R. and Bambawale, O. M. 2004. New strains of *Trichoderma* spp. strongly antagonistic against *Fusarium oxysporum* f. sp. *sesami*. *Indian J. Mycol. and Pl. Pathol.* **34**(1) : 107-109.

Sangle, U. R. Bambawale, O. M., Ahmad Nasim and Singh, S. K. 2003. Substrate and temperature requirements for sporulation of sub-tropical isolates of *Trichoderma* spp. *Ann. Pl. Protec. Sci.* **11** : 192-195.

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