

## Cultural Diversity of *Trichoderma* spp. Isolated from Different Agro-Climatic Regions

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### ABSTRACT

Based on the morphological characteristics, the 30 isolates of *Trichoderma* spp. were segregated into 10 groups. Under fluffy mycelial growth, five groups (I, II, III, IV and V) were categorized. The isolate Nos. 3, 9, 14 and 25 had been categorized in Group No. I based on the presence of fluffy mycelial growth with poor sporulation and presence of one ring and pigmentation. Isolate No. 8 was placed in Group II having fluffy mycelial growth with poor sporulation, presence of one ring and absence of pigmentation. Group No. III comprised of isolate Nos. 6 and 24 having same characters as in isolate No. 8 except there was no ring formation. Isolate Nos. 2, 4, 7, 23 and 20 were placed in Group No. IV with characteristic fluffy mycelial growth showing moderate to abundant sporulation, one ring formation and presence of pigmentation. Isolate Nos. 1, 10, 21 and 22 were placed in Group V having all the characters as in Group IV except more than one ring formation, heavy sporulation and absence of pigmentation. Under suppressed mycelial growth again five groups viz., Group VI, VII, VIII, IX and X were categorized. Group No. VI had isolate No. 19 with characters like poor sporulation, no ring formation and presence of pigmentation. The isolate No. 27 clustered in Group VII with characters as in Group No. VI except absence of pigmentation. Group No. VIII had the isolate Nos. 12, 17 and 26 with moderate sporulation, no ring formation and pigmentation. The isolate Nos. 5, 11, 13, 18, 28 and 29 grouped in Group IX with more than one ring and presence of pigmentation. Isolate Nos. 15, 16 and 30 have been categorized in Group No. X with abundant sporulation, more than one ring formation and presence of pigmentation.

**Key words:** *Trichoderma*, Mycelial, Pigmentation, Chlamyospore

### INTRODUCTION

*Trichoderma* is one of the novelties of nature that are common in soil root ecosystems throughout the world. They are present in all types of soil and other natural habitats specially those containing organic matter and have assumed great significance as a

biocontrol agent against several plant pathogens<sup>2,4</sup>. Mostly *Trichoderma* exhibits a preference for wet soils. However there are reports that individual species of *Trichoderma* exhibit different preferences for soil temperature and soil moisture.

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The species of *T. viride* are generally found in areas with low temperature whereas *T. harzianum* occur in warm climatic regions. *T. hamatum* and *T. koningii* have been reported to occur in diverse climatic conditions<sup>4</sup>. Diversity in *Trichoderma* spp. with regard to the cultural characters and antagonistic ability would depend mostly on the ecological condition of their habitats<sup>7</sup>. Recently, Singh et al<sup>8</sup>, noted that 35 species of *Trichoderma* are currently recognized and Nagamani et al<sup>3</sup>, in their comprehensive study characterized 13 species. *Trichoderma* species from India based on morphological and cultural characteristics. An attempt has been made to study variation in characters of it in the soils of different agricultural and horticultural crops spread across the different agro-climatic regions of the country.

#### MATERIALS AND METHODS

The morphological and cultural characteristics of 30 isolates of *Trichoderma* spp. isolated from soil samples of different locations of Patna, MokamaTal area (9) of Bihar, Nanded (1), Akola (2), Amravati (3), Nagpur (5), Aurangabad (1), Pune (1) of Maharashtra state, Sirsa (1), Kaul (2) of Haryana state, Jaspur (3) of Uttaranchal State, Indo-Israel farm IARI (1) and IARI field (1) Delhi, were collected. On the basis of morphological and cultural characteristics, all the 30 isolates have been grouped into 10 categories.

A repository of 30 *Trichoderma* spp. isolates has been developed in the present study. Some of these isolates are maintained at NCIPM, New Delhi and ICAR RCER, Patna derived from the previous collections done from three sources, viz., MokamaTal area in lentil crop (9) of Bihar, National Research Centre for Citrus, Nagpur (developed by U.R. Sangle and Late Dr. S.A.M.H. Naqvi) and one isolate from IARI, New Delhi. Eight strains were isolated from different agro-climatic regions of the country, viz., Nanded, Akola, Amravati, Nagpur, Shendurjanaghat, Pune, Kaul, Aurangabad, Sirsa and Jaspur. There is therefore good number of isolates of *Trichoderma* spp. were available for study on

the aspects of cultural variability (See the table below).

<i>Trichoderma</i> spp.	No. of isolate (s)
<i>T. harzianum</i>	10
<i>T. viride</i>	12
<i>T. hamatum</i>	03
<i>T. reesei</i>	03
<i>T. piluliferum</i>	01
<i>T. virens</i>	01

Throughout the study sparing over about 4 years isolation of *Trichoderma* species from different soil sample collected during the period of Jan Feb from different agricultural and horticultural crops growing in diverse agro-climatic regions were made following dilution plate method<sup>6</sup>. Martin's rose-bengal agar medium<sup>1</sup> was used for isolation of *Trichoderma* spp. Inoculated plates were incubated at 25<sup>0</sup>c under for 12 hrs alternate fluorescent light and darkness. The colonies (number) were counted after 5-7 days, purified and were confirmed as *Trichoderma* following Rifai<sup>5</sup>. The purified single spore cultures were maintained on PDA slants

To study the cultural variability of *Trichoderma* spp., 5 mm mycelial discs of each isolate were inoculated aseptically in the centre of 90 mm petri-plates (three replicates) containing 20 ml of PDA. Inoculated plates were incubated at 25±1<sup>0</sup>c and the observations of various colony characters were taken after every 24 hrs intervals upto 10 days.

#### RESULTS AND DISCUSSION

##### Cultural variability

Based on the observation of the conidia, phialides, colony texture, chlamyospore, conidiophore morphology the isolates were grouped into section. Morphological characteristics of 30 different isolates of *Trichoderma* spp. obtained from soil samples of various places were studied. On the basis of cultural characteristics all the 30 isolates of *Trichoderma* spp. have been grouped into I to X Groups. These groups are broadly divided on the basis of mycelial characters showing as fluffy mycelial and or suppressed mycelial growth, sporulation (good, moderate and

abundant), ring formation and presence or absence of pigmentation in mycelia. The five groups viz., I, II, III, IV and V have been categorized on the basis of fluffy mycelia growth character. The isolate Nos. 3, 9, 14 and 25 are categorized in Group No. I on the basis of fluffy mycelial growth depicting poor sporulation and presence of one ring and pigmentation. Isolate No. 8 has been placed in Group II having fluffy mycelial growth, poor sporulation, presence of one ring and absence of pigmentation. Group No. III comprised of isolate Nos. 6 and 24 having same characters as in isolate No. 8 except no ring formation. Isolate Nos. 2, 4, 7, 23 and 20 are placed in Group No. IV as these deciphered characteristic fluffy mycelial growths with moderate or abundant sporulation, one ring formation and presence of pigmentation. Isolate Nos. 1, 10, 21 and 22 are placed in Group V with having all the characters as in Group IV except more than one ring formation, abundant sporulation and absence of pigmentation. In earlier<sup>7</sup> have reported the similar results.

Under suppressed mycelial growth category five groups VI-X have been

designated. Group No. VI contain the isolate No. 19 with growth characters like poor sporulation, no ring formation and presence of pigmentation. The isolate No. 27 included Group VII possess all characters as in Group No. VI but no pigmentation. Group No. VIII possessing isolate Nos. 12, 17 and 26 with moderate sporulation, no ring formation and pigmentation. The isolate Nos. 5, 11, 13, 18, 28 and 29 repeat in Group IX showed more than one ring and presence of pigmentation. Isolate Nos. 15, 16 and 30 have been categorised in Group No. X because of heavy sporulation, more than one ring formation and presence of pigmentation (Tables 1 and 2).

Using the key of Rifai<sup>5</sup> in the earlier most of the strains isolated in these regions were identified as *T. harzianum*, *T. hamatum* and *T. viride*<sup>6</sup>. In fact, apart from *T. harzianum*, which, nevertheless accounted for the majority of isolates, a very interesting finding of this study was the coexistence of several species of *Trichoderma* (e.g. *T. harzianum*, *T. reesei*.) in the different ecosystem.

**Table 1: Cultural variability in different isolates of *Trichoderma* spp.**

Isolates	After 24 hrs		Transformed value		After 72 hrs		Transformed value		Growth rate	Isolate category
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD		
1. <i>T. harzianum</i>	15.67	1.15	4.00 <sup>i</sup>	0.14	90.00	0.00	9.50 <sup>a</sup>	0.00	very fast	V
2. <i>T. harzianum</i>	17.67	0.58	4.25 <sup>tg</sup>	0.07	85.00	0.00	9.23 <sup>c</sup>	0.00	Fast	IV
3. <i>T. viride</i>	15.00	0.00	3.92 <sup>ijk</sup>	0.00	85.00	0.00	9.23 <sup>c</sup>	0.00	Medium	I
4. <i>T. viride</i>	22.33	2.52	4.76 <sup>b</sup>	0.26	90.00	0.00	9.50 <sup>a</sup>	0.00	Fast	IV
5. <i>T. viride</i>	19.00	1.00	4.40 <sup>def</sup>	0.11	90.00	0.00	9.50 <sup>a</sup>	0.00	Medium	IX
6. <i>T. viride</i>	17.33	0.58	4.21 <sup>gh</sup>	0.07	90.00	0.00	9.50 <sup>a</sup>	0.00	Fast	III
7. <i>T. hamatum</i>	15.33	0.58	3.96 <sup>ij</sup>	0.07	90.00	0.00	9.50 <sup>a</sup>	0.00	Slow	IV
8. <i>T. viride</i>	16.00	0.00	4.05 <sup>hi</sup>	0.00	85.00	0.00	9.23 <sup>c</sup>	0.00	Slow	II
9. <i>T. viride</i>	15.00	0.00	3.92 <sup>ijk</sup>	0.00	85.00	0.00	9.23 <sup>c</sup>	0.00	Medium	I
10. <i>T. harzianum</i>	25.00	0.00	5.04 <sup>a</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	V

Table 1: contd. Cultural variability in different isolates of *Trichoderma* spp.

Isolates	After 24 hrs		Transformed value		After 72 hrs		Transformed value		Growth rate	Isolate category
	Mean	+SD	Mean	+SD	Mean	+SD	Mean	+SD		
11. <i>T. viride</i>	17.33	0.58	4.21 <sup>gh</sup>	0.07	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	IX
12. <i>T. hamatum</i>	14.67	0.58	3.88 <sup>ijk</sup>	0.07	75.00	0.00	8.67 <sup>e</sup>	0.00	Fast	VIII
13. <i>T. viride</i>	25.00	0.00	5.04 <sup>a</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Fast	IX
14. <i>T. reesei</i>	13.67	0.58	3.75 <sup>kl</sup>	0.08	75.00	0.00	8.67 <sup>e</sup>	0.00	Medium	I
15. <i>T. viride</i>	13.00	0.00	3.66 <sup>l</sup>	0.00	65.00	0.00	8.08 <sup>g</sup>	0.00	Fast	X
16. <i>T. hamatum</i>	14.00	0.00	3.79 <sup>ijkl</sup>	0.00	85.00	0.00	9.23 <sup>c</sup>	0.00	Medium	X
17. <i>T. harzianum</i>	19.33	1.53	4.44 <sup>de</sup>	0.17	90.00	0.00	9.50 <sup>a</sup>	0.00	Fast	VIII
18. <i>T. harzianum</i>	25.67	1.15	5.10 <sup>a</sup>	0.11	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	IX
19. <i>T. reesei</i>	18.00	0.00	4.29 <sup>efg</sup>	0.00	70.00	0.00	8.38 <sup>f</sup>	0.00	Medium	VI
20. <i>T. harzianum</i>	24.33	0.58	4.97 <sup>a</sup>	0.06	90.00	0.00	9.50 <sup>a</sup>	0.00	Fast	IV

Table 1: Cultural variability in different isolates of *Trichoderma* spp.

Isolates	After 24 hrs		Transformed value		After 72 hrs		Transformed value		Growth rate	Isolate category
	Mean	+SD	Mean	+SD	Mean	+SD	Mean	+SD		
21. <i>T. harzianum</i>	20.00	0.00	4.51 <sup>cd</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	V
22. <i>T. harzianum</i>	19.00	0.00	4.40 <sup>def</sup>	0.00	81.33	3.21	9.03 <sup>d</sup>	0.18	Fast	V
23. <i>T. harzianum</i>	21.00	0.00	4.62 <sup>bc</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	IV
24. <i>T. reesei</i>	22.00	0.00	4.73 <sup>b</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	III
25. <i>T. harzianum</i>	15.00	0.00	3.92 <sup>ijk</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	I
26. <i>T. viride</i>	25.00	0.00	5.04 <sup>a</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Very fast	VIII
27. <i>T. piluliferum</i>	0.67	0.58	0.99 <sup>m</sup>	0.32	7.67	0.58	2.81 <sup>h</sup>	0.10	Very slow	VII
28. <i>T. viride</i>	18.00	0.00	4.29 <sup>efg</sup>	0.00	87.00	0.00	9.34 <sup>b</sup>	0.00	Fast	IX
29. <i>T. viride</i>	15.00	0.00	3.92 <sup>ijk</sup>	0.00	90.00	0.00	9.50 <sup>a</sup>	0.00	Fast	IX
30. <i>T. virens</i>	19.00	0.00	4.40 <sup>def</sup>	0.00	85.00	0.00	9.23 <sup>c</sup>	0.00	Fast	X
<b>CD at 5%</b>	<b>0.15</b>				<b>0.06</b>					

\*Based on Table 2

Table 2: Grouping of growth characters of *Trichoderma* spp. on PDA

Character	Group No.	Isolate No.
<b>1) Fluffy mycelial growth</b>		
<b>1.1 With poor sporulation</b>		
1.1.i. One ring formation and pigmentation present	I	T3, T9, T14, T25
1.1.ii. One ring formation and pigmentation absent	II	T8
1.1.iii. No ring formation and pigmentation present	III	T6, T24
<b>1.2. With moderate / abundant sporulation</b>		
1.2.i. One ring formation and pigmentation present	IV	T2, T4, T7, T23, T20
1.2.ii. More than one ring formation and abundant sporulation but pigmentation absent	V	T1, T10, T21, T22
<b>2) Suppressed mycelial growth</b>		
<b>2.1 With poor sporulation</b>		
2.1. i. No ring formation and pigmentation present	VI	T19
2.1. ii. No ring formation and pigmentation absent	VII	T27
<b>2.2 With moderate sporulation</b>		
2.2. i. No ring formation and pigmentation absent	VIII	T12, T17, T26
2.2. ii. More than one ring formation and pigmentation present	IX	T5, T11, T13, T18, T28, T29
<b>2.3. With abundant sporulation</b>		
2.3. i. More than one ring formation and pigmentation present	X	T15, T16, T30

### CONCLUSION

Species of *Trichoderma* are diverse fungal microbial possess many qualities and they have great potential use in agriculture. The use of this genus has expanded worldwide as general plant protectants and growth enhancers. The genome of *Trichoderma* spp. has been extensively investigated and has proven to contain many useful genes, along with the ability to produce a great variety of expression patterns, which allows these fungi to adapt to many different environments and different variability on different ecosystems. The metabolomics of *Trichoderma* spp. are incredibly complex, especially in terms of secondary metabolites production but with the help of advanced molecular and proteomic approaches, it is possible to explore new pathways, novel functions of compounds produced by this genus and their potential applications.

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### REFERENCES

1. Martin, J.P., Use of acid, rose-bengal and Streptomycin in the plate method for

estimating fungi. *Soil Sci.*, **69**: 215-232(1950).

2. Mukhopadhyay, A.N. and Mukherjee, P.K., Fungi as Fungicides. *Inter Jour Trop. Pl. Dis.*, **14(1)**: 1-17 (1996).
3. Nagamani, A., Manoharachary, C., Agarwal, D.K. and Chowdhary, P.N., Monographic Contribution on *Trichoderma*. Associated Publishing Company. New Delhi (2002).
4. Papavizas, E.C., *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. *Ann. Rev. Phytopathol.*, **23**: 23-54 (1985).
5. Rifai, M.A., A revision of the genus *Trichoderma*. *Mycol. Papers. Imp. Mycol. Inst.* **116**: 1-56 (1969).
6. Sangle, U.R. and Bambawale, O.M., New strains of *Trichoderma* spp. strongly antagonistic against *Fusarium oxysporum* f. sp. *sesami*. *Indian J. Mycol. Pl. Pathol.*, **34(1)**: 107-109 (2004).
7. Sangle, U.R., Bambawale, O.M., Ahmad Nasim and Singh, S.K., Substrate and temperature requirements for sporulation of sub-tropical isolates of *Trichoderma* spp. *Ann. Pl. Protec. Sci.*, **11**: 192-195 (2003).
8. Singh, U.S., Zaidi, N.W., Joshi, D., Khan, T., John, D. and Bajpai, A., *Trichoderma*: A microbe with multifaceted activity. *Annu. Rev. Plant Pathol.* **3**: 33-75 (2004).