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Morphological and cultural variability of most widely used antagonist, *Trichoderma* spp.

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Abstract

Use of Biological control agents becomes key element of sustainable agriculture which maintain ecological balance and shows antagonism against many major problematic plant diseases of world. Among the all BCAs, *Trichoderma* species are most extensively studied species in fungal genera. In order to utilize full potential of *Trichoderma* species as an antagonist against target phytopathogenic fungi in several ways, it becomes essential to have systemic knowledge concerning to the behavior of these fungi. So present study carried out for characterization of an antagonist used for management of predominant soil borne fungal pathogens i.e. *Trichoderma* spp. These isolates of *Trichoderma* spp. were subjected to the cultural studies by growing on Potato dextrose agar plates and accordingly sorted them into groups of respective species on basis of macroscopic i.e. colony characters like growth rates, pustule formation, pigmentation showing defined characteristics for that respective species.

Keywords: *Trichoderma*, biocontrol agent, cultural variability, morphology

1. Introduction

Trichoderma belongs to phylum Ascomycetes, class Sordariomycetes, order Hypocreales, and family Hypocreaceae, represents a genus of asexually reproducing saprophytic fungi. In *Trichoderma* sexual stages (Perfect stages) are not known or rarely found and so reproduction is limited to the production of conidia. *Trichoderma* are beneficial opportunistic, a-virulent plant symbionts inhabiting in soil acts as natural antagonist against predominant soil borne fungal pathogens including fungal genera like *Fusarium*, *Sclerotium* and *Rhizoctonia*. The precise use of *Trichoderma* proven to improve root and plant growth and triggered induction of resistance in plants (Harman *et al.*, 2004) [6]. Despite of that, the most common and widely used biocontrol agent in India, species of *Trichoderma* genus are not well identified. For years, characterization of this most important and common biocontrol agent remained problematic due to variability in phenotypic characters. Isolates of *Trichoderma* spp. were subjected to the cultural studies by growing on Potato dextrose agar plates and accordingly sorted them into groups of respective species on basis of macroscopic i.e. colony characters like growth rates, pustule formation, pigmentation etc. showing defined characteristics of that respective.

2. Materials and Methods**2.1 Materials**

2.1.1 Biocontrol agent: *Trichoderma* isolates obtained from rhizospheric soils of western Maharashtra region.

2.1.2 Morphological characterization**2.1.2.1 Equipments**

Standard laboratory equipments like autoclave, hot air oven, BOD incubator, refrigerator, laminar air flow, research microscope, stereoscopic binocular, electronic balance machine, grinding machine, digital camera, pH meter and micro pipettes, etc. were used where ever necessary.

2.1.2.2 Chemicals

The chemicals, stains, other reagents, ready-made culture media (make: Hi-media), ingredients of culture media (Annexure I), etc. were obtained from the Department of Plant Pathology and Agricultural Microbiology, Rahuri.

2.2 Methods

2.2.1 Morphological and Cultural characterization of *Trichoderma* isolates

The morphological characteristics and growth rates of *Trichoderma* spp. isolates were studied on Potato dextrose agar (PDA) medium. Three replications were maintained for each isolate.

The petriplates were incubated in darkness at 28 ± 1 °C. The colonies were examined at 24 hrs intervals and colony radius was measured from the edge of inoculum plug after 7 days. The following observations on the growth rate and cultural characters of the isolates were recorded:

1. Colony diameter on PDA after 7 days
2. Colony colour
3. Conidial colour
4. Reverse colony colour
5. Colony edge
6. Conidial shape
7. Conidiation
8. Mycelial form
9. Chlamydo spores

The *Trichoderma* isolates were identified on the basis of cultural and microscopic features followed by the method of Subramanian (Subramanian, 1971)^[17].

3. Result and Discussions

3.1 Morphological and Cultural characterization of *Trichoderma* isolates

Trichoderma isolates (Trc-1, Trc-2, Trc-3, Trc-4, Trc-5, Trc-6, Trc-7, Trc-8, Trc-9, Trc-10, Trc-11, Trc-12, Trc-13, Trc-14, Trc-15, Trc-16, Trc-17, Trc-19 and Trc-20) were placed into different groups on the basis of their distinguishing characters. For the identification of *Trichoderma* spp. 3-7 days old culture grown on PDA was used. The *Trichoderma* strains were morphologically identified by using cultural characters like colony growth rate, colony colour, colony edge, growth pattern, mycelial form, mycelial colour and presence or absence of chlamydo spores etc.

Accordingly 20 *Trichoderma* isolates were grouped into 4 different *Trichoderma* spp. i.e. *T. hamatum* (6 isolates), *T. harzianum* (7 isolates), *T. koningii* (3 isolates) and *T. asperellum* (4 isolates). Microscopic examination was carried out according to Bissett (1984, 1991 a, b, c) classification method. The morphological characters of the *Trichoderma* spp. under research experimental studies (Table 1 a, b, c and d) were agree with the description given earlier by workers (Samuels, *et al.*, 1999; Shahid, *et al.*, 2013; Shah, *et al.*, 2012; Sriram, *et al.*, 2013)^[11, 14, 13, 16].

3.1.1 Colony characters

Colony characters of *Trichoderma* isolates were studied using 3-4 days old culture on PDA plates. All *Trichoderma* isolates grew well and formed conidia within 4 days. In colonies of isolates *viz.*, (Trc-1, Trc-3, Trc-4, Trc-6, Trc-9, Trc-12 and

Trc-19) dark green conidia were observed. Reverse colony colour of these isolates was colourless and effuse ring like zones were produced. These are the peculiar characters of *T. harzianum*. These isolates were identified as *T. harzianum*. In *T. hamatum*, colony colour was yellow to pea green and reverse colony colour was colourless to light yellow observed on PDA. In respect of zonation, on Potato dextrose agar isolates exhibited ring like zones. These characters observed in Trc- 7, Trc-8, Trc-10, Trc-14, Trc-15, and Trc-18 isolates. In isolates of *Trichoderma i.e.* Trc-2, Trc-11, Trc-16 and Trc-17, the colour of mature conidia was green. The colony colour of these isolates was mostly snow white green. The reverse colony colour was prominently cream and a ring like zones were produced. The isolates were found with chlamydo spores formation. On the basis of these observations isolates were identified as *T. asperellum*. In *T. koningii*, the conidia appeared to be dirty green and ring like zones with yellowish reverse colony colour on PDA was observed. These characters observed in Trc-20, Trc-13 and Trc-5 isolates.

3.1.2 Growth rate

In cultural characterization, these isolates were also identified on the basis of growth rate. The colony diameter of each *Trichoderma* isolate on PDA was recorded. The isolates of *T. harzianum* (Trc-1, Trc-3, Trc-4, Trc-6, Trc-9, Trc-12 and Trc-19) and *T. hamatum* (Trc- 7, Trc-8, Trc-10, Trc-14, Trc-15 and Trc-18) which showed 80-90 mm growth in 4 days after inoculation. The isolates of *T. koningii* (Trc-20, Trc-13 and Trc-5) showed 70-80 mm growth in 3 days. Whereas, *T. asperellum*, (Trc-2, Trc-11, Trc-16 and Trc-17) grew moderately slower 40-60 mm in 3 days.

3.1.3 Microscopic features

The observations recorded for growth characteristics were confirmed by microscopic studies using 40X and 100 X Olympus light microscope. The microscopic features of *Trichoderma* isolates were identified on the basis of shape and colour of conidia, mycelia form and presence of chlamydo spores. The colour of conidia of all *Trichoderma* were found to be green to dark green except *T. koningii* it was greyish green. Thus different intensities of green colours (green, dark green and greyish green) of mature conidia, observed on PDA plate. Shape of conidia, was more subglobose, in isolates of *T. harzianum* (Trc-1, Trc-3, Trc-4, Trc-6, Trc-9, Trc-12, Trc-19) compared to *T. asperellum* (Trc-2, Trc-11, Trc-16 and Trc-17) where it was subglobose with slightly ovoidal shape. The isolates of *T. koningii* (Trc-20, Trc-13, Trc-5) showed ellipsoidal oblong conidia; while isolates of *T. hamatum* (Trc- 7, Trc-8, Trc-10, Trc-14, Trc-15, Trc-18)) showed oblong to ovoidal conidia. Only four *Trichoderma* isolates *viz.* Trc-2, Trc-11, Trc-16 and Trc-17 showed production of chlamydo spores. On the basis of these observations isolates were confirmed as *T. asperellum*. Results of the present investigations on cultural and morphological characterization of *Trichoderma* isolates are in consonance with those reported earlier by several workers (Bissett, 1991; Samuels, *et al.*, 1999; Meraj-ul haq and Nandkear, 2012; Kumar *et al.*, 2013; Sriram *et al.*, 2013; Saravanan *et al.*, 2014; Ghutukade *et al.*, 2015 and Singh *et al.*, 2016)^[2, 11, 8, 7, 16, 12, 5, 15].

Table 1a: Morphological description used for the identification and characterization of *Trichoderma* spp. Isolates

Name of strain	Colony growth rate (mm)	IP (Days)	Colony colour	Reverse colony colour	Colony edge	Mycelia form	Mycelia colour	Conidiation	Conidia	Chlamydo spores
<i>T. harzianum</i> (Trc-1)	80-90	4	Whitish green to dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Dark green	Not observed
<i>T. asperellum</i> (Trc-2)	40-60	3	Green with snow whitish growth	Cream coloured to orange	Smooth	Floccose	Watery white	ring like zones	Sub-globose, slightly ovoidal, green	Observed
<i>T. harzianum</i> (Trc-3)	80-90	4	dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Dark green	Not observed
<i>T. harzianum</i> (Trc-4)	80-90	3	Whitish green to dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Dark green	Not observed
<i>T. koningii</i> (Trc-5)	70-80	3	Dirty green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	ring like zones	Ellipsoidal oblong, Greyish green	Not observed

Table 1b: Morphological description used for the identification and characterization of *Trichoderma* spp. Isolates

Name of strain	Colony growth rate (mm)	IP (Days)	Colony colour	Reverse colony colour	Colony edge	Mycelia form	Mycelia colour	Conidiation	Conidia	Chlamydo spores
<i>T. harzianum</i> (Trc-6)	80-90	4	Whitish green to dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Dark green	Not observed
<i>T. hamatum</i> (Trc-7)	70-80	3	Yellow to olive green	Colourless to light yellow	Smooth	Floccose	Watery white	Ring like zones	Oblong to ovoidal, green	Not observed
<i>T. hamatum</i> (Trc-8)	70-80	3	Yellow to pea green	Colourless to light yellow	Smooth	Floccose	Watery white	Ring like zones	Oblong to ovoidal, green	Not observed
<i>T. harzianum</i> (Trc-9)	80-90	3	Dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Dark green	Not observed
<i>T. hamatum</i> (Trc-10)	70-80	3	Yellow to pea green	Colourless to light yellow	Smooth	Floccose	Watery white	Ring like zones	Oblong to ovoidal, green	Not observed

Table 1c: Morphological description used for the identification and characterization of *Trichoderma* spp. Isolates

Name of strain	Colony growth rate (mm)	IP (Days)	Colony colour	Reverse colony colour	Colony edge	Mycelia form	Mycelia colour	Conidiation	Conidia	Chlamydo spores
<i>T. asperellum</i> (Trc-11)	40-60	3	Green centre with snow whitish growth	Cream coloured to orange	Smooth	Floccose	Watery white	ring like zones	Sub-globose, slightly ovoidal, green	Observed
<i>T. harzianum</i> (Trc-12)	80-90	4	Whitish green to green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Green	Not observed
<i>T. koningii</i> (Trc-13)	70-80	3	Dirty green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	ring like zones	Ellipsoidal oblong, Greyish green	Not observed
<i>T. hamatum</i> (Trc-14)	80-90	4	Yellow to pea green	Colourless to light yellow	Smooth	Floccose	Watery white	Ring like zones	Oblong to ovoidal, green	Not observed
<i>T. hamatum</i> (Trc-15)	70-80	3	Yellow to pea green	Colourless to Light yellow	Smooth	Floccose	Watery white	Ring like zones	Oblong to ovoidal, green	Not observed

Table 1d: Morphological description used for the identification and characterization of *Trichoderma* spp. Isolates

Name of strain	Colony growth rate (mm)	IP (Days)	Colony colour	Reverse colony colour	Colony edge	Mycelia form	Mycelia colour	Conidiation	Conidia	Chlamydo spores
<i>T. asperellum</i> (Trc-16)	40-60	3	Green with intermittent whitish growth	Cream coloured to orange	Smooth	Floccose	Watery white	ring like zones	Sub-globose, slightly ovoidal, green	Observed
<i>T. asperellum</i> (Trc-17)	40-60	3	Snow white to dark green	Cream coloured to orange	Smooth	Floccose	Watery white	ring like zones	Sub-globose, slightly ovoidal, green	Observed
<i>T. hamatum</i> (Trc-18)	70-80	3	Yellow to pea green	Colourless to Light yellow	Smooth	Floccose	Watery white	Ring like zones	Oblong to ovoidal, green	Not observed
<i>T. harzianum</i> (Trc-19)	80-90	4	Whitish green to dark green	Colourless	Wavy	Floccose to Arachnoid	Watery white	Effuse ring like zones	Sub-globose, Dark green	Not observed

<i>T. koningii</i> (Trc-20)	70-80	3	Dirty green	Yellowish	Smooth	Floccose to Arachnoid	Watery white	ring like zones	Ellipsoidal oblong, Greyish green	Not observed
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4. Conclusions

In the respective study, we isolated and identified 20 isolates of *Trichoderma*. Among them, isolates were grouped into 4 different *Trichoderma* spp. i.e. *T. hamatum* (6 isolates), *T. harzianum* (7 isolates), *T. koningii* (3 isolates) and *T. asperellum* (4 isolates). The isolates showed lot of cultural and morphological variabilities on PDA media and thus variation among the isolates was existed and confirmed.

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