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

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DOI: [https://doi.org/10.22271/chemi.2021.v9.i1b.11214](https://doi.org/10.22271/chemi.2021.v9.i1b.11214)

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 Sordrionymycetes, order Hypocreals, 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 symbiotants 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 species.

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 inoculums 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. Chlamydospores

The Trichoderma isolates were identified on the basis of their distinguishing features observed in Trichoderma spp. 3-7 days old culture grown on PDA. 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 chlamydospores 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 chlamydospores 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-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 chlamydospores 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-9, Trc-12 and Trc-19) showed ellipsoidal oblong conidia; while isolates of T. hamatum (Trc-7, Trc-8, Trc-10, Trc-14, Trc-15, and Trc-18) showed oblong to ovoidal shape. The isolates of T. koningii (Trc-20, Trc-13, Trc-5) showed elliptoid oblong conidia; while isolates of T. hamatum (Trc-7, Trc-8, Trc-10, Trc-14, Trc-15, and Trc-18) showed oblong to ovoidal conidia. Only four Trichoderma isolates viz.,Trc-2, Trc-11, Trc-16 and Trc-17 showed production of chlamydospores. 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).
### 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 | Chlamydomospores |
|----------------|-------------------------|-----------|---------------|-----------------------|-------------|--------------|---------------|-------------|----------|-----------------|
| *T. harzianum* (Trc-1) | 80-90 | 4 | Whitish green to dark green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Dark green | Not observed |
| *T. asperellum* (Trc-2) | 40-60 | 3 | Green with snow whitish growth | Cream coloured to orange | Smooth | Flocose | Watery white | ring like zones | Sub-globose, slightly ovoidal, green | Not observed |
| *T. harzianum* (Trc-3) | 80-90 | 4 | dark green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Dark green | Not observed |
| *T. harzianum* (Trc-4) | 80-90 | 3 | Whitish green to dark green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Dark green | Not observed |
| *T. koningii* (Trc-5) | 70-80 | 3 | Dirty green | Yellowish | Smooth | Flocose 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 | Chlamydomospores |
|----------------|-------------------------|-----------|---------------|-----------------------|-------------|--------------|---------------|-------------|----------|-----------------|
| *T. harzianum* (Trc-6) | 80-90 | 4 | Whitish green to dark green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Dark green | Not observed |
| *T. hamatum* (Trc-7) | 70-80 | 3 | Yellow to olive green | Colourless to light yellow | Smooth | Flocose | Watery white | Ring like zones | Oblong to ovoidal, green | Not observed |
| *T. hamatum* (Trc-8) | 70-80 | 3 | Yellow to pea green | Colourless to light yellow | Smooth | Flocose | Watery white | Ring like zones | Oblong to ovoidal, green | Not observed |
| *T. harzianum* (Trc-9) | 80-90 | 3 | Dark green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Dark green | Not observed |
| *T. hamatum* (Trc-10) | 70-80 | 3 | Yellow to pea green | Colourless to light yellow | Smooth | Flocose | 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 | Chlamydomospores |
|----------------|-------------------------|-----------|---------------|-----------------------|-------------|--------------|---------------|-------------|----------|-----------------|
| *T. asperellum* (Trc-11) | 40-60 | 3 | Green centre with snow whitish growth | Cream coloured to orange | Smooth | Flocose | Watery white | ring like zones | Sub-globose, slightly ovoidal, green | Observed |
| *T. harzianum* (Trc-12) | 80-90 | 4 | Whitish green to green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Green | Not observed |
| *T. koningii* (Trc-13) | 70-80 | 3 | Dirty green | Yellowish | Smooth | Flocose to Arachnoid | Watery white | ring like zones | Ellipsoidal oblong, Greyish green | Not observed |
| *T. hamatum* (Trc-14) | 80-90 | 4 | Yellow to pea green | Colourless to light yellow | Smooth | Flocose | Watery white | Ring like zones | Oblong to ovoidal, green | Not observed |
| *T. hamatum* (Trc-15) | 70-80 | 3 | Yellow to pea green | Colourless to Light yellow | Smooth | Flocose | 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 | Chlamydomospores |
|----------------|-------------------------|-----------|---------------|-----------------------|-------------|--------------|---------------|-------------|----------|-----------------|
| *T. asperellum* (Trc-16) | 40-60 | 3 | Green with intermittent whitish growth | Cream coloured to orange | Smooth | Flocose | Watery white | ring like zones | Sub-globose, slightly ovoidal, green | Observed |
| *T. asperellum* (Trc-17) | 40-60 | 3 | Snow white to dark green | Cream coloured to orange | Smooth | Flocose | Watery white | ring like zones | Sub-globose, slightly ovoidal, green | Observed |
| *T. hamatum* (Trc-18) | 70-80 | 3 | Yellow to pea green | Colourless to Light yellow | Smooth | Flocose | Watery white | Ring like zones | Oblong to ovoidal, green | Not observed |
| *T. harzianum* (Trc-19) | 80-90 | 4 | Whitish green to dark green | Colourless | Wavy | Flocose to Arachnoid | Watery white | Effuse ring like zones | Sub-globose, Dark green | Not observed |
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.

5. Acknowledgement
The authors thank Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413722, Maharashtra, India for providing all possible facilities for present study in partial fulfilment of Ph. D research work.

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