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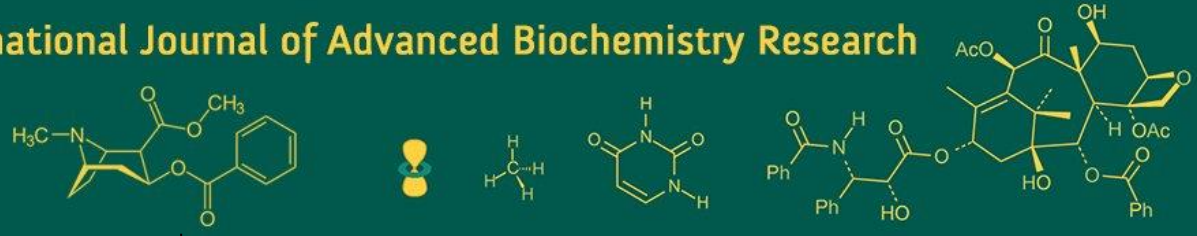

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Cultural and morphological characteristics of *Trichoderma* spp. and soil borne plant pathogens

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Abstract
The cultural characteristics and growth rates of four *Trichoderma* spp. *viz. Trichoderma asperellum, Trichoderma harzianum, Trichoderma reesei* and *Trichoderma hamatum* and three soil borne plant pathogens *viz. Fusarium oxysporum* f. sp. *vasinfectum, Sclerotium rolfsii* and *Rhizoctonia bataticola* were determined on Potato dextrose agar (PDA) medium. The colony diameter on PDA after 7 days, colony growth type, colony colour and pigmentation in the colony of the *Trichoderma* spp. and soil borne plant pathogens were recorded.

Keywords: ºC - Degree celcius, DAI - Days after incubation, *viz*., Videlicet (Namely), PDA - Potato Dextrose Agar, Spp./sp. – Species, F. - *Fusarium*

Introduction
The use of bio-agents having bio-control and plant growth promotion (PGP) activities have been considered as naturally and environmentally acceptable alternative to minimize the use of synthetic chemicals and their hazardous effects, and to provide protection to the plants against resident pathogen populations (Schipper *et al*. 1987, Lugtenberg *et al*. 2001) [20, 14]. Fungi are the most extensively researched group of biological control agents. (Weindling 1932) [24] Over 90 years ago, demonstrated the antagonistic nature of fungal species from the genus, *Trichoderma.*

The genus *Trichoderma* belongs to Domain – Eukaryota, Kingdom – Fungi, Division – Ascomycota, Phyllum - Ascomycetes, Class- Sordariomycetes, Order- Hypocreales and Family- Hypocreaceae. The genus *Trichoderma* is the most common saprophytic fungi in the rhizosphere and widely distributed in all types of soil and other diverse habitats (Hajieghrari *et al*. 2008) [6]. Rapid growth rate in culture and production of numerous spores that are varying shades of green characterize this fungus (Howell 2003, Shalini and Kotasthane, 2000) [8, 19].

The *Trichoderma* spp. are known to exhibit mycoparasitism, antibiosis, enzyme secretion, competition and induction of systemic resistance in plants as a means to inhibit the growth and multiplication of its target fungi (Benitez *et al*. 2004) [4]. The *Trichoderma* spp. produce numerous biologically active compounds, including cell wall degrading enzymes (Harman, 2000, Vinale *et al*. 2008) [7, 23], antifungal metabolites (Susanne and Markus, 2007) [22], volatile (Michrina *et al*. 1995) [15] and non- volatile compounds (Benitez *et al*. 2004) [4] that impede colonization of pathogens in the rhizosphere of the plant, which help in reducing/inactivating the pathogens population in the soil environment. There have been numerous recent attempts to use *Trichodema* spp. against soil borne pathogens such as *Sclerotinia, Fusarium, Pythium* and *Rhizoctonia* species (Elad *et al*. 1983; Jager *et al*. 1991; Ashrafizadeh *et al*. 2005) [5, 9, 1]. Among these, several species of *Trichoderma* are well documented mycoparasites and have been used successfully against certain pathogenic fungi. The *T. harzianum, T. viride, T. virens, T. hamatum, T. roseum* and *T. koningii* are the species that most often used in biological control of plant pathogens (Papavizas and Lumbsden 1980; Papavizas, 1985) [16, 17].

Materials and Methods

The cultural characteristics and growth rates of *Trichoderma* spp. and soil borne plant pathogens were determined on Potato dextrose agar (PDA) medium. A 5 mm diameter disc from the actively growing edge of a fresh colony of the isolates was cut by using a sterile cork borer and placed in a 90 mm Petri dish, containing 20 ml of sterilized PDA medium aspectically. Three replications were maintained for each isolate. The Petri dishes were incubated at 28±2 °C. The colonies were examined at 24 h intervals and colony radius was measured from the edge of the inoculum after 7 days. The following observations on growth rate and cultural characteristics of the *Trichoderma* spp. and soil borne plant pathogens were recorded:

1. Colony diameter on PDA after 7 days.
2. Colony growth type.
3. Colony colour.
4. Pigmentation in the colony.

Results and Discussion

Cultural and morphological characteristics and study of *Trichoderma* spp.: Morphological characters of four *Trichoderma* spp. *viz. Trichoderma asperellum, Trichoderma harzianum, Trichoderma reesei* and *Trichoderma hamatum* with respect to radial growth and colony characters were studied on PDA. The radial growth (mm) of *Trichoderma* spp. was measured at 7th days after inoculation and data is presented in table 1 and figure 1.

The basic for taxonomical studies is morphological characterization which even through is a long established technique still today it has importance. The four species of *Trichoderma viz. T. asperellum, T. harzianum, T. reesei* and *T. hamatum* were examined for colony morphology study. The isolates were grown on PDA later for 3 to 7 days as pure culture and the various mycelial colony and conidial characters of different species of *Trichoderma* were recorded. The cultural and morphological characters were based on ten characters included here *viz.* radial growth, colony growth, colony colour, pigmentation, mycelium colour, phialide shape, conidiation, branching of conidiophore, conidial colour, conidial shape etc. Table 1 and figure 2.

The *T. asperellum* and *T. reesei* showed 90 mm radial growth 7 days after inoculation with similar conidiophore i.e. concentric zone and moderately branched with green colour conidia, however globose to subglobose conidia observed in *T. asperellum* and ellipsoid conidia in *T. reesei.* The ring like conidiation, highly branched conidiophore seen, subglobose conidia were observed in *T. harzianum*. The frequently branched, white cottony conidiophore with green colour, globose conidia were noted in *T. hamatum*. The colony growth of *T. asperellum* was subaerial and disperse, milky white to dark green colour and sigmoid or hooked type phialide noted. The major difference between *T. harzianum, T. reesei* and *T. hamatum* were their colony growth pattern, colony colour, pigmentation, phialide and shape. White colour pigmentation were noted in *T. asperellum* and *T. harzianum*, dark yellow and yellow colour pigmentation recorded in *T. reesei* and *T. hamatum* respectively.

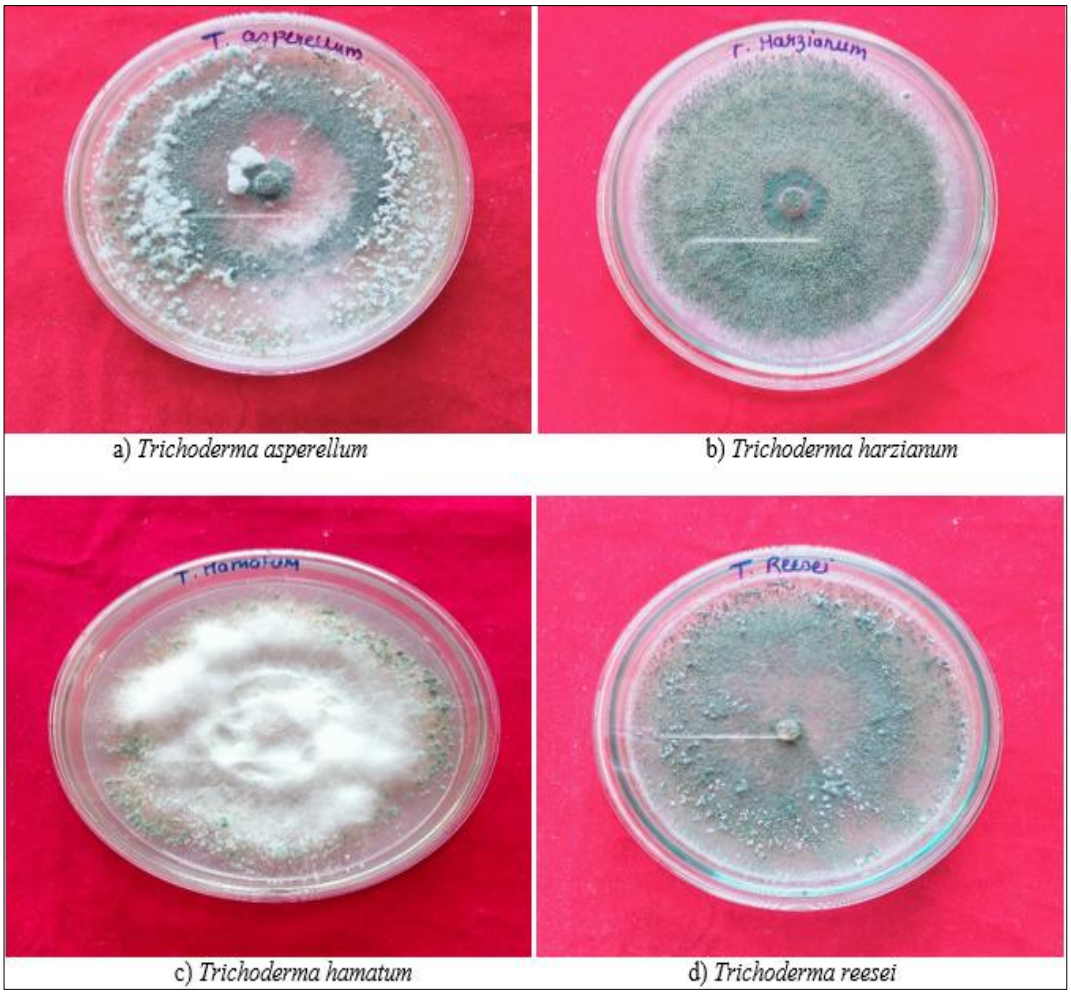
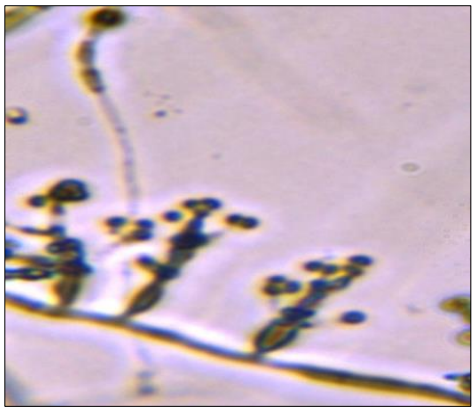


Fig 1: Pure culture of four *Trichoderma* spp.

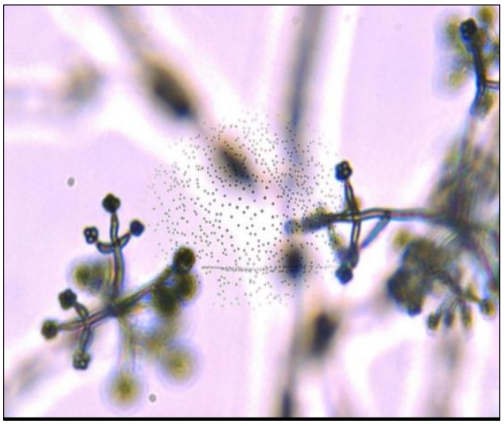
Table 1: Morphological characteristics of *Trichoderma* spp.

Sr. No.	Isolates	Radial growth (mm) at 7 DAI	Colony characters					Conidiophore		Conidia	
			Colony growth type	Colony colour	Pigmentation	Mycelium colour	Phialide shape	Conidiation	Conidiophore branching	Conidial colour	Conidial shape
1.	*T. asperellum*	90.00	Sub aerial and disperse	Milky white to dark green	White colour	White	Sigmoid or hooked	Concentric zone	Moderately branch	Green	Globose to subglobose
2.	*T. harzianum*	89.67	flat and disperse	Green to dark green	White colour	Watery white	Globose	Ring like zones	Highly branched	Green	Subglobose
3.	*T. reesei*	90.00	White cottony and disperse	white to grayish green	Dark yellow colour	Greenish-grayish	Globose to subglobose	Concentric zones	Moderately branched	Green	Ellipsoide
4.	*T. hamatum*	90.00	White cottony earlier & light green after	White to light green	Yellow colour	Greeish	Frequently paired, globose	White cottony growth	Frequently branched	Green	Globose

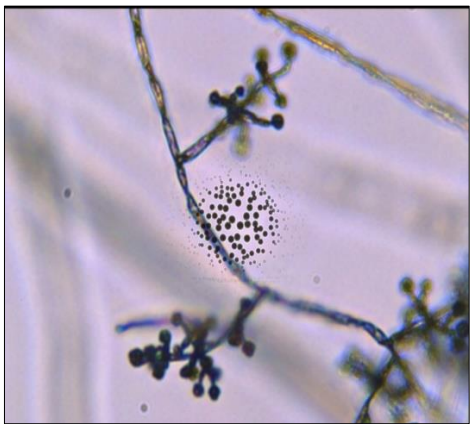
Trichoderma asperellum



Trichoderma harzianum



Trichoderma hamatum



Trichoderma reesei

Fig 2: Microscopic view of four *Trichoderma* spp.

The present results are in agreement with Kumar and Sharma (2016) [12], who were isolated *Trichoderma* spp. The isolates belonging to *T. harzianum* were analogous in colony colour, culture smell, mycelial colour, conidiation, conidial shape, conidial wall and conidial colour. The isolates of *T. viride* showed certain similarity in colony colour, colony edge, culture smell, conidiophore branching, conidial wall, conidial colour and chlamydospores.

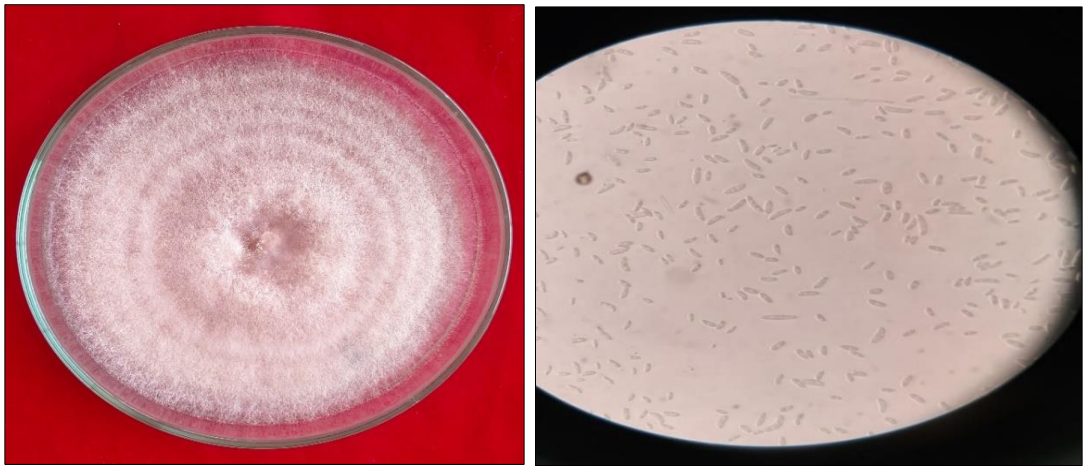
Soesanto *et al*. (2011) [21] *Trichoderma* spp. (*T. harzianum* and *T. pseudokongii)*, morphological characteristic study was done by observation of fungal colony growth on PDA and MEA media and studied the colony colour as velvetinous with white and dark green flocouse surface along with scattered green patches and yellow to green pigmentation on PDA medium.

Study of morphological characteristics and growth rate of soil borne plant pathogens

Morphological characters of three soil borne plant pathogens *viz*. *Fusarium oxysporum* f. sp. *vasinfectum*, *Sclerotium rolfsii* and *Rhizoctonia bataticola* with respect to radial growth and colony characters were studied. All these soil borne plant pathogenic fungi were grown on PDA for 7 days and colony growth, mycelial and conidial characters were recorded.

a) *Fusarium oxysporum* f. sp. *vasinfectum*

After seven days of incubation at 28 + 2 ºC temperature, the pathogen *Fusarium oxysporum* f. sp. *vasinfectum* showed white cottony with fluffy growth which turn pink or purple on the reversed side of peteiplates. The colony diameter of the pathogen recorded at 7 DAI was 85-90 mm. The microscopic examination of *Fusarium oxysporum* f. sp. *vasinfectum* under light microscope at 100 X revealed two kinds of spores i.e. microconidia and macroconidia. Microconidia were produced on short monophilllides singly, Zero to one septate and oval to ellipsoid/kidney shaped. The macroconidia were falcate shaped divided into three to five septate slightly curved or pointed at end.



Fusarium oxysporum f. sp. *Vasinfectum* Microscopic view microconidia & macroconidia

Fig 3: Microscopic view *Fusarium oxysporum*

These findings were congruent with Asif *et al*. (2023) [3], reported that white cotton appearance with fluffy growth of *Fusarium oxysporum* f. sp. *vasinfectum* on PDA medium which turn pink or purple colour on the back of petriplate, colony diameter ranged from 80 to 85 mm on seven days of inoculation in PDA at 25 ºC. Also they reported the microscopic observation of *Fusarium oxysporum* f. sp. *vasinfectum* under light microscope at 100 X were produced three kinds of spores *viz*. microconidia, macroconidia and chlamydospores which are in agreement with present study. All these factors also corresponded to the critical morphological features of *F. oxysporum* species cited by Leslie and Summerell (2006) [13] Amini and Sidovich (2010) [2] reported white cottony to pink often with purple colour of medium, hence therefore identified as *Fusarium oxysporum* f. sp. *vasinfectum.*

b) *Sclerotium rolfsii*

The cultural and morphological characterization of fungal pathogen *Sclerotium rolfsii* was studied by growing on PDA plates. After 7 days of incubation at a temperature of 28 + 2ºC, the soil borne fungal plant pathogen *Sclerotium rolfsii* showed 90 mm radial growth on petriplate. The fungal mycelium was first silky white in colour later turned to dull white with radial spreading given fan like appearance. The microscopic examination of *Sclerotium rolfsii* under light microscope at 100 X revealed the aerial, hyaline thin walled, septate hyphae with profusely branched mycelium showing clamp connection. The 10 to 15 old culture of the fungus produced small mycelial knots which later turned to mustard seed like sclerotia, appeared deep brown later brownish black, shiny, hard and spherical to irregular in shape.

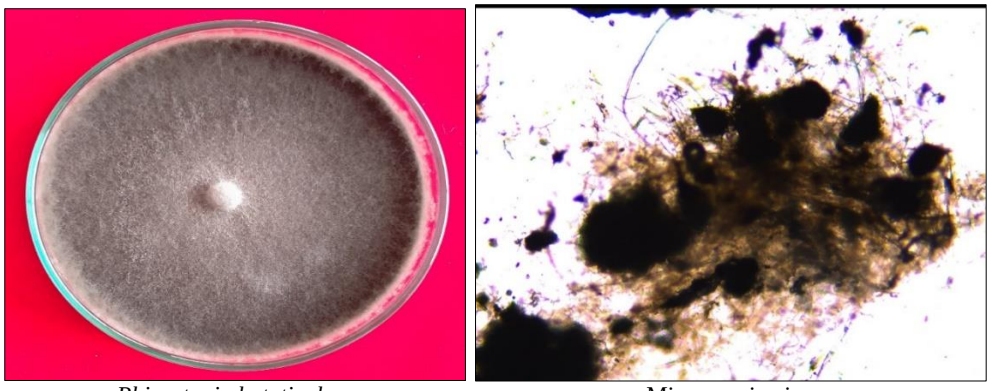
Sclerotium rolfsii Microscopic view

Fig 4: *Sclerotium rolfsii* Microscopic view

The present findings were congruent with previous studies also reported that *Sclerotium rolfsii* produced silky white mycelium initialy later turns to dull white with radial spreading given fan like appearance, septate hyphae with profusely branched mycelium and when fungus attained maturity deep brown or brownish black, shiny, hard and spherical to irregular shaped small mycelial knots were formed. (Kumar *et al*. 2014) [11], thus the fungus under present investigation was confirmed as *Sclerotium rolfsii*.

c) *Rhizoctonia bataticola*

After seven days of incubation at a temperature 28+2 ºC, the fungal pathogen shown greyish to black colour colonies with fluffy and velvety texture on petriplate containing with potato dextrose agar medium. The colony diameter of the pathogen was 90 mm after 7 days of inoculation and the fungi was fast growing aerial mycelium of grey to black colour as high as to touch the lid cover of the culture plates later lie flat to bottom of petriplates as it ages. The microscopic examination of *Rhizoctonia bataticola* under light microscope at 100 X, the mycelium shows characteristics features of *Rhizoctonia bataticola* as septate, right angle branching of mycelium, constriction of hyphae near the point of origin produced black to brown sclerotia with age, which are spherical, oblong to irregular shapes.



Rhizoctonia bataticola Microscopic view

Fig 5: *Rhizoctonia bataticola* Microscopic view

The colony characters and morphological characters of mycelium and sclerotia were in agreement with the description of Ram and Singh (2018) [18] and Jainpur *et al*. (2020) [10]. Thus the fungus under present investigation was confirmed as *Rhizoctonia bataticola* (Tauh.).

Conclusion

The study conducted an in-depth analysis of Trichoderma species focusing on their cultural and morphological characteristics. Through detailed observations of radial growth, colony morphology, pigmentation, conidial shape, and other key features, distinctions between *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma reesei*, and *Trichoderma hamatum* were identified. Notably, T. *asperellum* and T. *reesei* exhibited similar radial growth and conidial characteristics, while T. *harzianum* and T. *hamatum* showed distinct colony growth patterns and pigmentation. These findings underscore the importance of morphological characterization in taxonomic studies, providing valuable insights into the diversity and characteristics of Trichoderma species, which have implications for various fields including agriculture, biotechnology, and environmental management.

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