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Cultural and Morphological Characterization of *Trichoderma* spp. Associated with Green Mold Disease of *Pleurotus* spp. in Kashmir

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ABSTRACT

Various micro fungi associated with *Pleurotus* spp. were isolated from Mushroom Research and Training Center (M.R.T.C) in SKAUST, Srinagar. Among various fungi collected, the most predominant were those of *Trichoderma* spp. A total of 49 isolates of *Trichoderma* spp. were collected during oyster mushroom cultivation. On the basis of cultural and morphological characteristics, *Trichoderma* isolates were classified into three species, namely *Trichoderma harzianum, Trichoderma viride* and *Trichoderma pseudokoningii*. The predominant specie was *Trichoderma harzianum* (n = 19) followed by *Trichoderma viride* (n = 17) and *Trichoderma pseudokoningii* (n = 13). All the three species were distinct from each other in the characteristics such as mycelial growth rate, colony appearance, shape of conidia and conidiophores and branching pattern of phialides. This study has found *Trichoderma harzianum* to be the main cause of green mold disease of *Pleurotus* spp.

Key words: Microfungi, predominant, cultivation, oyster, mycelial

INTRODUCTION

Trichoderma spp. are soil inhabiting filamentous fungi, belongs to the genus *Hypocrea*. During the last decades, *Trichoderma* spp. have been reported to be responsible for causing green mold disease of *Pleurotus* mushroom (Steiner *et al*., 1997). In India, green mold disease in mushrooms caused by *Trichoderma* spp., was not of much significance to the mushroom industry (Sharma *et al*., 1999). But in the past decade, green mold has become a rather dangerous disease of mushroom industry. This disease causes complete crop failures in cases of severe infections. The pathogen inhibits the growth of mushrooms and in severe infections, the basidiocarps are not produced from contaminated beds (Yu, 2001; Park *et al*., 2005).

Cultivation of mushrooms is popular in India in recent days. Among the cultivated mushrooms, *Pleurotus sajor-caju* has several advantages that can be easily exploited by farmers for cultivation. This mushroom is very easy to cultivate. It does not need any composting like button mushroom. The genus *Pleurotus* secrete various enzymes specific for digesting lignocellulose materials. In Kashmir mushroom houses, commercial production of this mushroom has been affected badly by many vagaries of nature viz., pests and diseases that affect its production and productivity. Among the fungal pathogens, *Trichoderma* species are the most common (Oh *et al*., 2003). The symptoms of Green mold produced by *Trichoderma* spp. are characterized by systemic browning of caps

followed by drying of fruiting bodies (Szarka and Lukacs, 1988). The main symptoms of green mold are the appearance of greenish patches in the oyster mushroom substrates, green fungal sporulation in spawn bottles. *Trichoderma* spp. inhibits the growth of mushroom and causes complete crop losses. However, the occurrence and diversity of *Trichoderma* spp. associated with green mold of *Pleurotus* mushroom production have not been well studied.

The present study was undertaken to identify and characterize *Trichoderma* spp. present in commercial *Pleurotus* mushroom substrates based on cultural and morphological characteristics of *Trichoderma* species in *Pleurotus* mushroom substrate.

MATERIALS AND METHODS

Sample collection: Samples of *Pleurotus* mushroom bags with green mold growth were collected from Mushroom Research and Training Centre, Division of Plant Pathology, SKAUST-K, Shalimar, Srinagar, during the period from September, 2007 to November, 2007. The sampling included paddy straw bags with or without visual growth of *Pleurotus* spp. Samples were also collected from green mold infected spawn bottles. The fungi were then sub-cultured on fresh Potato Dextrose Agar (PDA) medium for identification and biotype characterization. Representative isolates from the samples were preserved for further studies. All cultures were maintained on PDA slants at 5°C.

Cultural and morphological observations: The characteristics of *Trichoderma* spp. like colony appearance and sporulation pattern were examined from cultures grown on six media: potato dextrose agar (PDA), cornmeal dextrose agar (CMD), malt extract agar (MEA), Czapek's dox agar (CZDA), Saboraud dextrose agar (SDA) and Nutrient agar (NA) at 28°C for 4 days. Three Petri dishes (9 cm) each containing 25 mL of media were used for each isolate. For observing colony characteristics and growth rate, inocula were taken from the actively growing margin of 4 days culture, grown on PDA. A 7 mm mycelial disc was placed at center of all Petri dishes. The dishes were kept for incubation at 28°C. Radial growth were measured at 24 h intervals until colony covers the whole petri dish. All micro morphological data were examined on cultures grown on PDA for five days at 28°C. The microscopic examination and measurements of conidiophores and conidia were made from slide preparations stained with lactophenol-cotton blue.

RESULTS

A total of 49 isolates of *Trichoderma* spp. were isolated during the cultivation of *Pleurotus* spp. in M.R.T.C-SKAUST-K. On the basis of the cultural and morphological characteristics, the *Trichoderma* isolates could be classified into three groups. On the basis of descriptions given by Gams and Bissett (1998), each group was identified as *Trichoderma harzianum* (38.7%), *Trichoderma viride* (34.6%) and *Trichoderma pseudokoningii* (26.5%). *Trichoderma harzianum* was the most common species found during the cultivation of *Pleurotus* spp. (Table 1).

Table 1: Occurrence of *Trichoderma* spp. during *Pleurotus* spp. cultivation

	Trichoderma spp.		
Source	*T. harzianum*	*T. viride*	*T. pseudokoningii*
Paddy straw	10	9	7
Spawn bottles	9	8	6
Total (%)	19 (38.7)	17 (34.6)	13 (26.5)

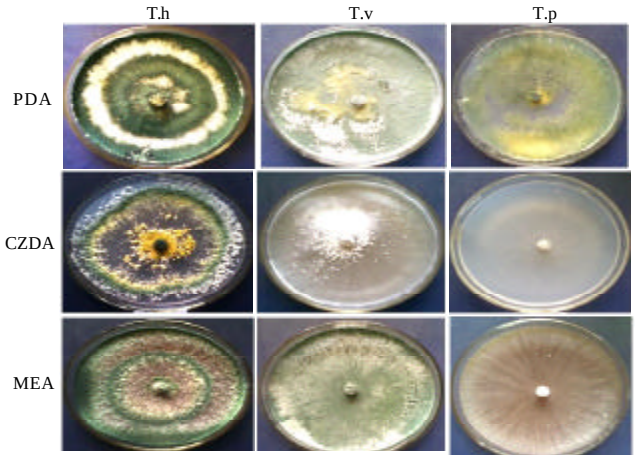

Fig. 1: Colony appearance of three different species of *Trichoderma* grown for 96 h at 28°C on PDA, CZDA and MEA, *T. harzianum* (T.h), *T. viride* (T.v) and *T. pseudokoningii* (T.p)

Cultural characteristics: Cultural characteristics comprising growth rate, colour and colony appearance were examined. These characteristics were regarded as taxonomically useful characteristics for *Trichoderma* (Samuels *et al*., 2002).

On PDA, *T. harzianum* formed 1-2 concentric rings with green conidial production. The conidia production was denser in center then towards the margins. *T. viride* appears to be a bit granular on PDA, with green conidia distributed throughout. An irregular yellow zone without conidia was present around the inoculum. Some white pustules were also found growing on the green mat of conidia. *T. pseudokoningii* formed white mycelia with no conidial formation (Fig. 1).

On CZDA, *T. harzianum* shows a single green and yellowish concentric ring with a cluster of yellow conidia around the point of inoculum. Some white conidia appear to grow scarcely towards the edges. *T. viride* on CZDA show light green and yellowish conidia scattered throughout the plate. There were no concentric rings formed by *T. viride* on CZDA. *T. pseudokoningii* on PDA forms white and transparent mycelia with little or no conidia production (Fig. 1).

On MEA, *T. harzianum* show white cottony mycelium with green conidiation. Two concentric rings were formed, one just touching the margin and the other around but away from the inoculum. Green conidia dispersed on the whole plate but appear dense near the rings. The light green conidia of *T. viride* were formed on the whole plate with more conidia towards the margins and towards the inoculum. No conidia formation and concentric rings were formed by *T. pseudokoningii* except the white mycelia cover the Petri dish (Fig. 1). The growth of *T. viride* was faster on MEA than the other two species, showing complete growth on 3rd day.

On CMD, 1-2 concentric rings were formed by *T. harzianum*. Green and whitish conidia of *T. harzianum* were more or less restricted to concentric rings. The growth of *T. viride* appear somewhat granular or powdery on CMD. No concentric rings were found. Light green and whitish aggregates of conidia were found. *T. pseudokoningii* form white mycelia with 1-2 concentric rings, with little white conidia dispersed near rings (Fig. 2).

On SDA, *T. harzianum* form cottony white mycelium with dark green conidiation towards the margins. A single cottony concentric ring was found around the inoculum. The mycelium of *T. viride* was not visible as light green conidia cover the entire media. A single concentric ring was

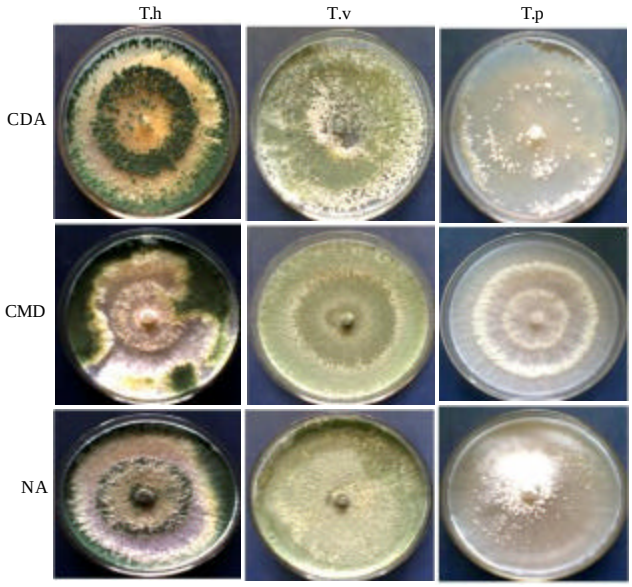

Fig. 2: Colony appearance of three different species of *Trichoderma* grown for 96 h at 28°C on CMD, SDA and NA, *T. harzianum* (T.h), *T. viride* (T.v) and *T. pseudokoningii* (T.p)

found around the point of inoculum. *T. pseudokoningii* on SDA, form white cottony mycelium through out the colony with no conidia formation. Two concentric rings, one towards the inoculum and the other away from the inoculum were found (Fig. 2). The growth of *T. pseudokoningii* was much slower on SDA, covered the petri dish on 5th day.

On NA, *T. harzianum* form cottony white and light yellowish mycelium of floppy growth. A single concentric ring of conidial production was formed away from the inoculum. Dark green conidia appear around inoculum and towards the periphery as well. *T. viride* on NA appear granular with light green and yellowish conidia dispersed through out the colony. No concentric rings were formed by *T. viride* on NA. *T. pseudokoningii* on NA form transparent mycelium with little white conidia scattered around the inoculum. No concentric rings were formed by *T. pseudokoningii* on NA (Fig. 2).

The three different species of *Trichoderma* show different cultural characteristics and exhibited different growth rates on six media at same temperature of 28°C. *T. viride* grew faster on all media followed by *T. harzianum* and then by *T. pseudokoningii*.

Micro morphological characteristics: While examining the five days culture of *Trichoderma harzianum, Trichoderma viride* and *Trichoderma pseudokoningii* grown on PDA, the following micro morphological differences were observed (Table 2). Conidia of *Trichoderma harzianum* (2.8×2.6 μm) were globose to subglobose. The conidia of *T. viride* (3.0×2.8 μm) were globose where as there was no conidia formation by *T. pseudokoningii* in five days old culture. Conidia were observed only after 7 days in *T. pseudokoningii*. Conidia of *T. pseudokoningii* (2.4×2.6 μm) were smaller than those of the other species. Conidia were pale green incase of *T. pseudokoningii* where as those of *T. harzianum* and *T. viride* were of light green colour.

Table 2: Morphological characteristics of *Trichoderma* spp. isolated from *Pleurotus* spp.

	Conidia		Phialide	
Trichoderma species	Shape	Size (µm)	Shape	Size (µm)
Trichoderma harzianum	Globose to subglobose	2.8×2.6	Flask shaped	5.0×2.6
Trichoderma viride	Globose	3.0×2.8	Slender	3.0×6.2
Trichoderma pseudokoningii	Oblong ellipsoidal	2.4×2.6	Elongated	8.0×2.6

Phialides were flask shaped in *T. harzianum*. Phialides of *T. viride* were also flask shaped but shorter than those of *T. harzianum*. Phialides of both *T. harzianum* and *T. viride* were arranged in divergent groups of 2-4. Phialides of *T. pseudokoningii* mostly arise singly and laterally and the whole conidiophore system appear singly rather than pyramidal incase of *T. harzianum* and *T. viride*.

DISCUSSION

Trichoderma spp. are responsible for causing green mold of *Pleurotus* mushroom (Hatvani *et al*., 2007). It is one of the serious disease of *Pleurotus* mushroom, causing severe economic losses to the mushroom farmers. In the present study, attempts were made to identify *Trichoderma* spp. associated with the green mold disease of this mushroom using cultural and morphological characteristics. *Trichoderma* isolates from green mold of *Pleurotus* mushroom were identified as three distinct species, viz., *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma pseudokoningii*. Among these three species, *T. harzianum* was the most common species, causing green mold disease in *Pleurotus* mushroom cultivation. These findings are as per those found by Castle *et al*. (1998).

In this study, colony appearance, colony colour, colony growth rate, colony morphology, conidial and phialide morphology and size could separate all the three species of *Trichoderma*. Colonies formed by *T. harzianum* somewhat resembles *T. viride* in appearance and morphology. Both the species form darker colonies with sufficient conidiation, while as colonies formed by *T. pseudokoningii* were almost whitish with little or no conidiation. *T. harzianum* show much pigmented mycelial growth than *T. viride*. *T. pseudokoningii* shows no pigmentation at all. Pigmentation in *T. pseudokoningii* was observed after 7-8 days. The faster growth rate was exhibited by *T. viride*, which was followed by *T. harzianum* on all media. *T. harzianum* show floppy growth on all media except CZDA. In comparison to the growth of *T. harzianum, T. viride* and *T. pseudokoningii* form mycelia adhered to the media. The conidia produced by *T. harzianum* and *T. viride* somewhat resembles each other. The length of phialides of *T. pseudokoningii* was longer than rest of the two species. All these descriptions were in conformity as per given by Gams and Bissett (1998).

On the basis of these results, the three species of *Trichoderma* could be classified, however additional phenotypic and genotypic characters should be evaluated for effective control of green mold of *Pleurotus* mushroom.

CONCLUSION

This study helped to identify the devastating weed (*Trichoderma* spp.) in *Pleurotus* mushroom cultivation. Growing the three species, *T. harzianum*, *T. viride* and *T. pseudokoningii* on different media helped us to know the growth pattern, sporulation pattern and various cultural and morphological characteristics, exhibited by different species of *Trichoderma*, causal agent of green mold infection.

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