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MYCOFLORA ASSOCIATED WITH OYSTER MUSHROOM (Pleurotus ostreatus) CULTIVATION AT M.R.T.C IN KASHMIR

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ABSTRACT

Mycoflora associated with Oyster mushroom (Pleurotus spp.) at Mushroom Research and Training Center (M.R.T.C) - SKAUST-K were studied. Around 11 fungi were isolated from Paddy straw used, spawn bottles, fully spawn-run straw and directly from the basidiocarps (fruiting bodies).The predominant Microfungi isolated from straw comprises of Aspergillus flavus, Aspergillus niger, Penicillium spp, Rhizopus spp and Trichoderma harzianum. Microfungi found in infected bottles comprises of Alternaria, Cephalosporium, Penicillium spp, Penicillium mineoluteum, Trichoderma viride and Trichoderma pseudokoninjii. Microfungi which occur predominantly on spawn-run straw and fruiting bodies were Penicillium spp., Trichoderma viride, Trichoderma harzianum and Fusarium oxysporum. Out of various microfungi isolated, the most dominant and devastating species were those of Trichoderma spp. and Penicillium spp., causing Green mold of Oyster mushroom (Pleurotus spp).

Key words: Bottles, Fruiting bodies, Microfungi, Predominant, Species.

I.INTRODUCTION

About 2000 species from more than 30 genera are regarded as prime edible mushrooms, but only about 80 species are grown experimentally, 40 cultivated economically, around 20 cultivated commercially and only 4-5 are produced on an industrial scale (Chang, 1990). Only three edible mushrooms, *Agaricus bisporus* (white button mushroom) *Volvariella* spp. (paddy straw mushroom) and *Pleurotus* spp. (oyster mushroom or dhingri – local name) are in commercial or semi-commercial cultivation in India. Jammu and Kashmir state comprises area of diverse climatic zones ranging from sub-tropical to temperate and alpine regions (Munshi and Ghani, 2003).

Among commercially cultivated mushrooms, *Pleurotus* species commonly known as Oyster mushroom, is extensively cultivated throughout the world and contributed more than 24.1 per cent of total world production (3.772 million metric tonnes in 1990) of mushroom (Munshi and Ghani, 2003). The Oyster mushroom

Pleurotus sp. is cultivated in many countries both in sub-tropical and temperate regions of the world. Oyster mushroom production has increased at a rapid rate worldwide in the last few years (Royse, 2013). An attractive feature of this group of mushroom is that they can utilize a large variety of agricultural waste products (Stamets, 2000) and transform the ligno cellulosic biomass into food of high quality, flavor and nutritive value. This mushroom can grow at a wide range of temperatures also (Baysal, *et al*. 2003). Oyster mushroom plays an important role in managing organic wastes whose disposal has become a problem (Das and Mukherjee, 2007).

Pleurotus spp. are found abundantly in valley, Drass (Ladakh district) and Jammu. Three species are commonly available in J&K State – *Pleurotus fossulatus* (Cooke) Sacc., *Pleurotus flabellatus* (Berk and Br.) Sacc. and *Pleurotus mambranacens* Massee. Commercial cultivation of *Pleurotus* specie was introduced in state by number of organisation since long and a good amount of reasonable work has been done on various aspects of this mushroom in SKUAST-K. Its cultivation in the valley has increased manifold due to its easy cultivation on different agro wastes and also because of its medicinal importance. Oyster mushroom cultivation is normally practiced on paddy and wheat straw. These straw substrates get heavily contaminated with harmful fungi, resulting in a partial and total failure of the mushroom cultivation. Weed fungi associated with *Pleurotus* spp. cultivation, compete for nutrients and impair the mushroom yield (Chhata and Thakore, 2010). Some fungal and bacterial pathogens have been reported to parasitize *Pleurotus* fruiting bodies and causing economic losses upto 76% (Suman and Sharma, 2005). Attempts were made by different workers to manage these fungal competitor moulds and get increased yields (Chakarvaty *et al.* 1982; Vijay and Sohi, 1987). Microfungi like *Trichoderma*, *Aspergillus* and *Penicillium* were identified as important competitors of *Pleurotus* spp. (Senthilpandia *et al.* 1996). The most dominant and devastating species were those of *Trichoderma* spp. (Thakur *et al*. 2001) causing a disease called Green mold. Over 30 species of fungi cause green mold in mushroom cultivation (Cha, 2004). The information on competitor moulds prevalent in mushroom house during cultivation of *Pleurotus* spp. is lacking. Therefore, the present study was carried out to isolate and identify other mycoflora occurring during the cultivation of *Pleurotus* spp. in Mushroom Research and Training Center (M.R.T.C) at SKAUST-K.

II.MATERIAL AND METHODS

Microfungi were collected at various stages of Oyster mushroom (*Pleurotus ostreatus*) cultivation in M.R.T.C–SKAUST-K during the period from September 2007 to November 2007. The *in vitro* studies were carried out in Microbiology Laboratory of Environmental Science Department, SKAUST-K. Various microfungi were isolated during the cultivation of oyster mushroom:

III.MYCOFLORA FROM DRY PADDY STRAW

The substrate used for the cultivation of Oyster mushroom was Paddy straw, as it was easily available in the campus. In the first stage, 4 dry paddy straw pieces (2 cm long) were taken for the mycoflora study. Straw pieces were introduced separately in 100ml flasks having 50 ml sterilized water. The flasks were vigorously shaken for 30 min to get homogenous suspensions. The suspension of 2 ml was poured in each petri dish (90mm) having 20ml Potato dextrose agar medium (PDA), containing 0.7 g of streptomycin per litre. The plates were gently rotated to ensure uniform distribution of inoculums, on the agar film. Three replications were

maintained, means the same inoculum in three plates. The petri dishes were kept for incubation at 27°C, till the fungal growth was visible. When the petri dishes were covered with different microfungi, they were subcultured individually on sterilized PDA slants using sterilized needle.

IV.MYCOFLORA FROM SPAWN BOTTLES

In the 2nd stage, infected spawn bottles were selected for mycofloral study. Five bottles were selected for the study. Out of five bottles, two were infected with dark green mycelia, one bottle with light green powdery growth, one with black to brownish mycelia and the fifth one, was not infected. All the bottles were labelled as B1, B2, B4, B5 & B3. The samples were obtained by dilution and plating of sampled material on rose benegal agar and also by dust sedimentation on the rose benegal agar medium. The hyphae of the growing fungi were transferred on PDA, amended with 0.7g streptomycin. In case of B3, the white mycelial thread on the wheat grains, was directly inoculated in the centre of petri dishes. Three replications were maintained for each bottle sample. All the petri dishes were kept for incubation at 27°C. The petri dishes were observed for the mycoflora on 5th day after incubation. The fungal colonies were then subcultured onto the sterilized PDA slants and labelled.

V.MYCOFLORA FROM POLY BAGS

During the cultivation process of *Pleurotus*, various microfungi were collected from the straw before spawn-run and fully spawn-run polythene bags. The samples were taken from the diseased bags. The straw pieces (3 cm) were directly placed on the sterilized PDA dishes under aseptic conditions. The plates were incubated at 27°C for 4 days. The fungal colonies in the petri dishes were subcultured by transferring the hyphal threads to sterilized PDA slants.

VI.MYCOFLORA FROM INFECTED FRUITING BODIES

Samples were also collected from the fruiting bodies especially from the pileus of basidiocarp. The diseased mushrooms were collected in polybags. A 5 mm flesh disc was cut from infected sporophore with the help of sterilized cork borer and inoculated on PDA Petri dishes under aseptic conditions. Three replications were maintained and then were incubated at 26 $\pm$ 1˚C for five days. Microfungi obtained were purified by hyphal tip method (Tutte, 1969).

The microfungi isolated from above samples were maintained on PDA slants. The slants were plugged properly, labelled and then stored at 4°C for identification.

VII.RESULTS

Mycoflora in Agar slants were identified microscopically. A total of 11 microfungi were isolated (Table 1). Dry paddy straw harboured maximum mycoflora. Microfungi isolated from dry paddy straw include *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp, *Rhizopus* spp and *Trichoderma harzianum*. Microfungi were also isolated from infected spawn bottles. Most of the bottles were infected with green spores (Fig 3). Mycoflora isolated from five bottles (B1, B2, B3, B4 & B5) was examined under microscope. Microfungi recorded from

spawn bottles include: microfungi isolated from B1 were *Penicillium* spp, *Trichoderma pseudokoninjii* & *Trichoderma viride.* B2 showed the presence of *Penicillium* spp *& Trichoderma viride*. *Trichoderma viride* & *Cephalosporium* were isolated from B3. B4 was dominated by *Penicillium* spp only. *Alternaria & Penicillium mineoluteum* were isolated from B5. *Penicillium mineoluteum* on PDA form small round colonies with pink to light reddish pigmentation towards the base of Petri dish. Paddy straw before spawn-run comprises of *Penicillium* spp and *Trichoderma harzianum. Trichoderma viride* and *Trichoderma harzianum* dominated the fully spawn-run straw. Microfungi isolated from the basidiocarps (fruiting bodies) were *Fusarium oxysporum, Penicillium* spp and *Trichoderma viride.*

Infected spawn bottles harboured maximum mycoflora followed by dry paddy straw. Out of 11 microfungi isolated, *Trichoderma* spp and *Penicillium* spp dominated almost every substrate and fruiting bodies as well.

VIII.DISCUSSION

The genus *Pleurotus,* though relatively new to mushroom industry, has gained much popularity world over because of its easy cultivation. The species of *Pleurotus* secrete an arsenal of enzymes specific for the digestion of lignocellulose materials. Unfortunately this mushroom is subjected to many vagaries of nature *viz*, pests and diseases that adversely affect its production and productivity.

Various microfungi were isolated from substrate (paddy straw), infected spawn bottles and basidiocarps. The maximum mycoflora was obtained from Dry paddy straw which is in conformity with the findings of Meera and Tewari, 1989. Microfungi like *Aspergillus flavus, Aspergillus niger*, *Penicillium* spp., *Rhizopus* spp. and *Trichoderma harzianum* were isolated from paddy straw. Microfungi isolated from infected spawn bottles include: *Penicillium* sp, *Trichoderma pseudokoninjii, Trichoderma viride, Cephalosporium Alternaria* & *Penicillium mineoluteum*. Microfungi like *Fusarium oxysporum*, *Penicillium* spp. and *Trichoderma viride* were isolated from the basidiocarps (fruiting bodies).

Among all microfungi isolated, *Trichoderma* spp. and *Penicillium* spp. were obtained from both substrate and fruiting bodies as well. Among the fungal pathogens, *Hyphomycetous* fungi including *Trichoderma* are the most common. Oh, *et al.* 2003 also reported the presence of *Trichoderma* spp. during his study. Morris, *et al.* (1995) also isolated *Trichoderma* species, namely *Trichoderma harzianum, Trichoderma viride, Trichoderma longibrachiatum* and *Trichoderma koningii* from air, swab and compost samples. They reported that *Trichoderma harzianum* was the most commonly isolated species. *Trichoderma* spp. are the most devastating fungi, responsible for causing a serious disease called Green mold in *Pleurotus* spp.

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(Fig 1) Mycoflora growing on straw before spawn-run.

(Fig 2) Mycoflora on fruiting bodies & primordia

(Fig 3) Infected spawn bottle

Table 1. Mycoflora associated with various substrates of Oyster mushroom cultivation.

Fungi isolated (Scientific name)	**Source**

Aspergillus flavus	Dry paddy straw
Aspergillus niger	Dry paddy straw
Rhizopus spp	Dry paddy straw
Trichoderma harzianum	Dry paddy straw
Penicillium spp	Dry paddy straw

	Spawn bottles
Penicillium spp, T.pseudokoninjii & T.viride.	B1
Penicillium spp & T.viride.	B2
T. viride & Cephalosporium.	B3
Penicillium spp.	B4
Alternaria & Penicillium mineoluteum.	B5

Penicillium spp	Straw(no spawn-run)
T.harzianum	Straw(no spawn-run)
T.harzianum	Spawn-run straw
T.viride	Spawn-run straw

Fusarium oxysporum .	Basidiocarps
Penicillium spp	Basidiocarps
T.viride	Basidiocarps