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Antagonistic relationships of *Agaricus* spp. and *Pleurotus* spp. with *Trichoderma harzianum* isolates

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ABSTRACT

The major species of mushrooms grown in India are white button mushroom, oyster mushroom and paddy straw mushroom. One of the most common and destructive diseases in mushroom cultivation is the green mould of which *Trichoderma* spp. induce significant quantitative and qualitative losses in the mushroom cultivation. In the present study, isolates of pathogenic fungi *Trichoderma harzianum* and edible mushrooms (*Agaricus campestris*, *Pleurotus ostreatus*, *P. florida* and *P. flabellatus*) are paired in all possible combination in dual culture experiments to understand the antagonistic interactions of these fungi have on each other *in vitro*. Cases of green mould were reported to be caused by *Trichoderma harzianum* in Mushroom industries developing in Jabalpur, M.P. Dual culture experiments of *Trichoderma harzianum* isolates with all the host mushrooms revealed that isolates of *Trichoderma harzianum* and the test host mushrooms are strongly antagonistic. In the total dual culture experimental set up 31.25% pairing shows deadlock on mycelial contact and 68.7% pairing shows overgrowth of pathogenic fungi on mushroom host after formation of initial deadlock.

INTRODUCTION

One of the most common and destructive diseases in mushroom cultivation is the green mould which is mainly caused by different species of *Trichoderma*, *Penicillium* and *Aspergillus*. Among these moulds, *Trichoderma* spp. induces significant quantitative and qualitative losses in the mushroom cultivation [8]. Green mold is characterized by large areas of dense hyphal network in mushroom compost or casing materials, followed by sporulation. *Trichoderma* colonized in mushroom compost competes with mushroom mycelium for space and nutrients and results in large areas of the growing beds not producing mushroom fruit bodies [4,3,7].

In the present study, pathogenic fungi *Trichoderma harzianum* isolates and edible mushrooms (*Agaricus* spp. and *Pleurotus* spp.) are paired in all possible combination in dual culture experiments to understand the antagonistic interactions of these fungi have on each other *in vitro*.

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Table 1. Results of dual culture experiment of four edible mushrooms with four isolates of fungal pathogen *Trichoderma harzianum* (A14, A15, A16, and A17).

Pathogenic fungi	Edible mushroom			
	Pleurotus ostreatus [14]	*Pleurotus florida* [11.5]	*Pleurotus flabellatus* [14]	*Agaricus campestris* [4]
A14 (*Trichoderma harzianumRifai*) [11.5]	C_{A1}	C_{A1}	C_{A1}	A
A15 (*Trichoderma harzianumRifai*) [9]	C_{A1}	A	C_{A1}	A
A16 (*Trichoderma harzianumRifai*) [11.5]	C_{A1}	C_{A1}	C_{A1}	A
A17 (*Trichoderma harzianumRifai*) [11.5]	C_{A1}	C_{A1}	C_{A1}	A

[1]*Antagonism index values are being represented in square brackets.*

MATERIALS AND METHODS

Survey in the month of February, shows that white button mushroom and oyster mushroom are mostly cultivated in Jabalpur district under environmental conditions. Diseased mushroom from various mushroom cultivation unit of Jabalpur, M.P., India were collected in sterile polythene bags and carried to the laboratory for the further studies.

Isolation and maintenance of the pathogens

Pieces of mycelium taken from green mold affected area of each sample were aseptically placed on 2% malt extract agar media (MEA) using a sterilized needle. The plates were incubated at room temperature (27^0C) until the fungal growth was visible. The fungi were then sub cultured on fresh Potato Dextrose Agar medium with streptomycin [5].

Maintenance of pure culture of edible mushroom

Pure cultures of different edible mushrooms (*Agaricus campestris* MTCC972, *Pleurotus ostreatus* MTCC142, *P. florida* MTCC9193 and *P. flabellatus* MTCC1799) were brought from the culture bank Microbial Type Culture Collection, Institute of Microbial Technology, Chandighar, India and maintained in PDA slants at 25^0C until the growth was visible.

Identification of pathogens

The isolated microorganisms were identified on the basis of its cultural and morphological characteristics, which was also confirmed from National Fungal Culture Collection of India, Agharkar Research Institute, Pune, Maharashtra, India.

Dual culture experiment

Competitive interactions between *Agaricus campestris, Pleurotus ostreatus, P. florida* and *P. flabellatus* and 4 isolates *Trichoderma harzianum* were studied in dual-culture experiments on PDA in Petri dishes. In each dish, two 2- mm diameter of mycelial disks, one from a mushroom colony and one from isolated fungal pathogens, were placed on the agar surface 30 mm apart. Mushroom host was inoculated 3 days before to the inoculation of pathogens. The mushrooms and pathogenic fungi were paired in all possible combinations. Three replicates were prepared for each pairing. A rating scale with 3 types (A, B and C) and 4 sub-types (C_{A1}, C_{B1}, C_{A2} and C_{B2}) of reactions was used for each fungus, where: A, deadlock, mutual inhibition, in which neither organism was able to overgrow the other after mycelial contact; B, deadlock at a distance i.e. without mycelia contact; C, replacement, overgrowth without initial deadlock; C_{A1}, partial replacement after initial deadlock; C_{A2}, complete replacement after initial deadlock; C_{B1}, partial replacement after initial deadlock at a distance; C_{B2}, complete replacement after initial deadlock at a distance. The following score was assigned to each type or sub-type of reaction: A=1; B=2; C=3; C_{A1}=3.5; C_{B1}=4; C_{A2}=4.5; C_{B2}=5. The antagonism index (AI) was calculated for each fungal species using the formula: $AI = \Sigma n \times i$. Where n= number (frequency) of each type or sub-type of reaction; i= corresponding score [2].

RESULTS AND DISCUSSION

Four pathogenic fungi were isolated and named as A14, A15, A16, and A17 from green mold infected areas in mushroom compost which were identified as *Trichoderma harzianum* Rifai (NFCCI3363, NFCCI3368, NFCCI3370 and NFCCI3364 respectively) on the basis of their microscopic and macroscopic (morphology) studies. Pure culture of edible mushroom hosts namely *Agaricus campestris*, *Pleurotus ostreatus, P. florida* and *P. flabellatus* were prepared in slants of PDA medium.

For competitive interaction studies between edible mushrooms and isolates of *Trichoderma harzianum*, dual-culture experiments were performed on PDA in Petri dishes.

In the total dual culture experimental set up 31.25% pairing shows deadlock on mycelial contact. Dense mycelium in the interaction zone was produced by both, mushrooms and *Trichoderma harzianum* isolates. Overgrowth of pathogenic fungi on mushroom host after formation of initial deadlock was shown by 68.7% pairing (Table 1).

On the basis of calculated AI values *Pleurotus ostreatus, Pleurotus florida* and *Pleurotus flabellatus* belong to moderately active mushrooms of group II (AI=15-10) and *Agaricus campestris* is least active against *Trichoderma harzianum* isolates (Table 1).

Dual culture experiments of *Trichoderma harzianum* isolates with all the host mushrooms revealed that *Trichoderma harzianum* and the test host mushrooms are antagonistic. Formation of deadlock on mycelial contact was recorded in 31.25% pairing, suggesting that some form of recognition response was involved. Such result was also obtained by Rayner and Webber [6].

Overgrowth of pathogenic fungi on mushroom host after formation of initial deadlock in 68.7% pairing suggests recognition response as well as overgrowth may be due to lysis and parasitism on large scale or in some cases overgrowing mycelium may simply smother the overgrown mycelium. Similar results were reported earlier [6]. They also say that though replacement is common in basidiomycotina interaction, its mechanism remains unclear.

AI is relatively constant for each species and can be used for bio-ecological characterization. Establishing the AI is the first step in screening for physiological and biological activity [1].

Cases of green mould were reported, isolation and identification of the fungal pathogen indicates presence and prevalence of *Trichoderma harzianum* in Jabalpur. Proper control measures need to be practiced to prevent crop loss. Out of the four mushroom species tested in this study, *Pleurotus ostreatus, Pleurotus florida* and *Pleurotus flabellatus* can be cultivated well in Jabalpur as compared to *Agaricus campestris,* with regards to its antagonistic behavior to *Trichoderma harzianum* isolates.

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