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Interactions between *Agaricus campestris* the edible mushroom and *Aspergillus* spp. the pathogenic fungus through dual-culture technique

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Abstract

In India majority of the mushroom holdings are lacking adequate compost preparation, pasteurization and proper environmental control facilities, which lead to the development of various diseases and pests sufficiently to a level to cause considerable yield loss. It is therefore very important for the mushroom growers that they should know the importance of diseases and microbial competitors to grow mushrooms successfully and profitably.

In the present study, pathogenic fungi, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* were paired with edible mushroom, *Agaricus campestris*, in all possible combination in dual culture experiments. In the total dual culture experimental set up 33.33% pairing shows deadlock on mycelial contact and 66.67% pairing shows deadlock at a distance.

Keywords: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Agaricus campestris*, dual culture experiment

The major species of mushrooms grown in India are white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* species) and paddy straw mushroom (*Volvariella volvacae*) of these, white button mushroom contributes about 90% of the total product (Lidhoo and Agarwal 2006; Khader 2001).

In India majority of the mushroom holdings are lacking adequate compost preparation, pasteurization and proper environmental control facilities, which lead to the development of various diseases and pests sufficiently to a level to cause considerable yield loss. It is therefore very important for the mushroom growers that they should know the importance of diseases and competitors and should understand the importance of hygiene to grow mushrooms successfully and profitably.

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* (Sharma et al. 2007).

In the present study, pathogenic fungi, species of *Aspergillus* and edible mushrooms (*Agaricus campestris*) were paired in all possible combination in dual culture experiments to understand the antagonistic interactions of these fungi have on each other *in vitro*.

Materials and methods

Collection of natural competitor

During the survey in the month of January (2012), it was observed that white button mushroom is widely prevalent in Jabalpur district under natural environmental conditions. Fungal infected mushroom compost from various mushroom cultivation units of Jabalpur district, M P, India were collected in sterile polythene bags and carried to the laboratory for further studies.

Isolation and identification of the fungal 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 27°C, until the fungal growth was visible. The fungi were then sub cultured on fresh Potato Dextrose Agar medium with streptomycin (Jayalal and Adikaram 2007).

Fungal isolates were identified on the basis of its cultural and morphological characteristics. The identity of the pathogens were also confirmed from National Fungal Culture Collection of India, Agharkar Research Institute, Pune, Maharashtra, India.

Maintenance of pure culture of edible mushroom - *Agaricus campestris*

Pure cultures of the edible mushroom *Agaricus campestris* in Lyophilized form was brought from the culture bank Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India and was maintained in PDA slants and petriplates at 25°C until the growth was visible.

Dual culture experiment

Competitive interactions between edible mushroom *Agaricus campestris* and fungal pathogens namely - *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus*

Table 1. Macroscopic characters of the isolated pathogenic fungi

Pathogenic Fungus		Macroscopic characters		
		Colour	Texture	Shape/ margin
A9	*Aspergillus niger*	Black colour colony	Powdery	Circular
A13	*Aspergillus flavus*	White mycelium with green spore	Powdery	Circular with white border
A19	*Aspergillus fumigatus*	White mycelium, greenish blue spore	Cottony/ rough	Circular narrow white border

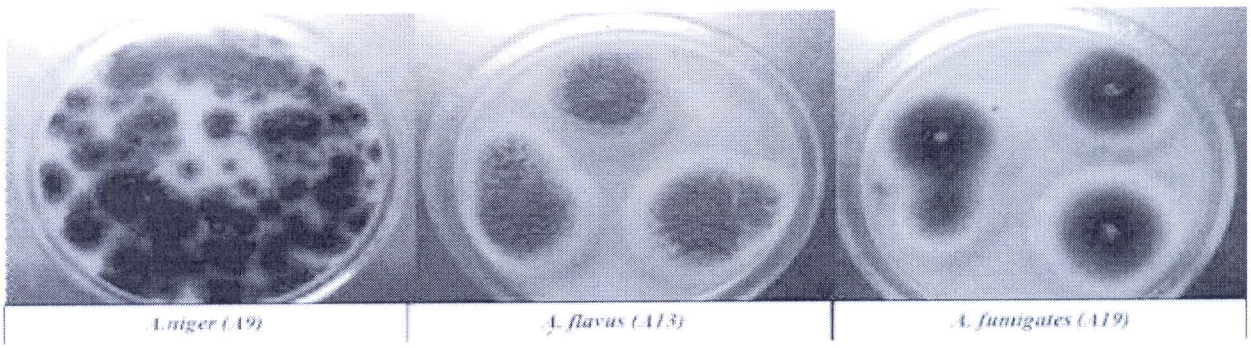

Fig 1. Pure culture of fungal pathogens

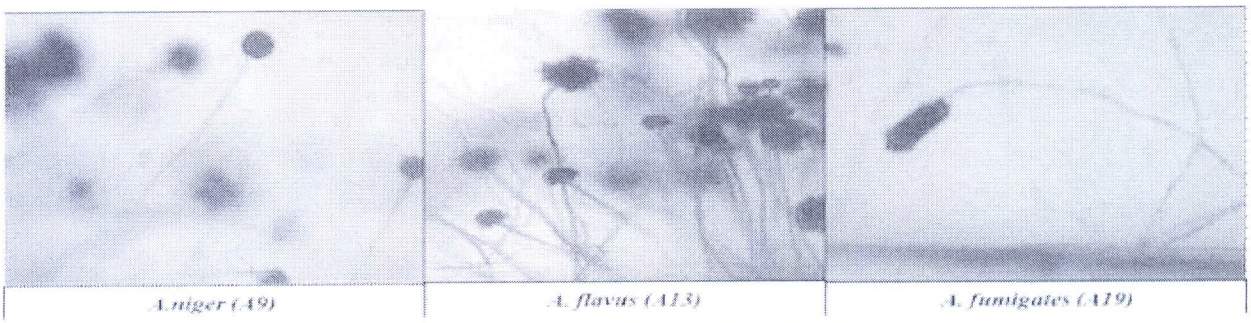

Fig 2. Micro photographs of isolated fungal pathogens

flavus were studied in dual-culture experiments on PDA in Petri dishes. In each dish, two 2- mm diameter of mycelial disks, one from the mushroom colony and one from the fungal pathogen, were placed on the agar surface 30 mm apart. Mushroom host was inoculated 3 days before to the inoculation of pathogens. 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: $AI = \sum n \times i$ where n= number (frequency) of each type or sub-type of reaction; i= corresponding score (Badalyan et al. 2002; Badalyan et al. 2004).

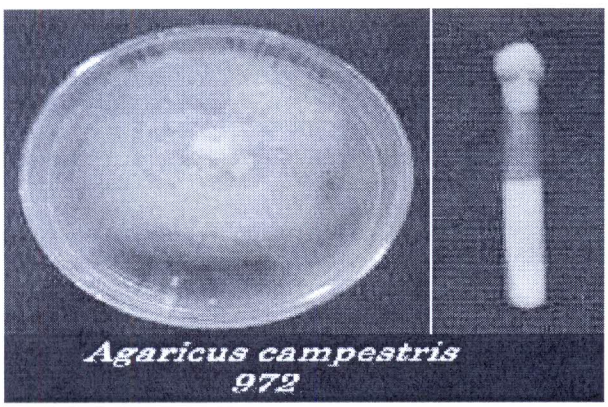

Fig 3. Pure culture of edible mushroom *Agaricus campestris*

Result and discussion

Three pathogenic fungi were isolated and named as A9, A13 and A19 from green mold infected areas in mushroom compost (Fig 1) which were identified as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*, respectively (Fig 2 and Table 1).

Pure culture of edible mushroom host *Agaricus campestris* (MTCC No. 972) was prepared in Petri plates and slants of PDA medium (Fig 3).

On performing competitive interaction studies between host *Agaricus campestris* and fungal pathogen *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* in dual-culture experiments, following results were obtained (Fig 4 and Table 2).

Dual culture experiments of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* (A9, A13 and A19) with the host edible mushroom *Agaricus campestris* revealed that all the *Aspergillus* isolates and the test host mushrooms were strongly antagonistic. In the total dual culture experimental set up 33.33% pairing shows deadlock on mycelial contact. Dense mycelium in the interaction zone was produced by both, mushroom and *Aspergillus niger*, when there was deadlock, suggesting that some form of recognition response was involved. Such result was also obtained by Rayner and Webber (1984) in their interaction studies with different host and mycoparasite. 66.67% pairing shows deadlock

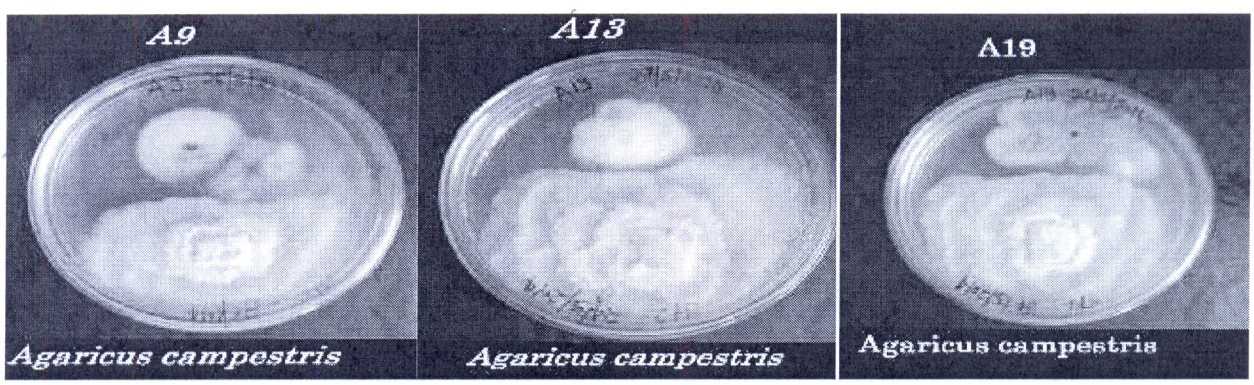

Fig 4. Interaction between the host *Agaricus campestris* and the fungal pathogens namely *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* in dual culture experiment.

Table 2. Results of dual culture experiment of edible mushroom *Agaricus campestris* with fungal pathogens*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* (A9, A13 and A19)

Pathogenic fungi	Edible Mushroom *Agaricus campestris* [5]
A9 (*Aspergillus niger* gr.) [1]	A
A13 (*Aspergillus flavus* gr.) [2]	B
A19 (*Aspergillus fumigatus* Fresen.) [2]	B

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

at a distance which suggests that fungal pathogens produce volatile/non-volatile metabolite(s) active against the mushroom (Rayner and Webber 1984).

On the basis of calculated AI values *Agaricus campestris* was found to be less active against tested *Aspergillus* isolates. 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 (Badalyan 1998).

This study reveals the growing interest of mushroom cultivation and its scope in Jabalpur district of Madhya Pradesh, India. Cases of green mould were reported, isolation and identification of the fungal pathogen indicates presence and prevalence of *Aspergillus* sp. in Jabalpur area.

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