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BIOLOGICAL EFFICACY OF MYCOFLORA ISOLATED FROM MUSHROOM SUBSTRATE AGAINST PATHOGENIC FUNGI

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ABSTRACT

Four fungi isolates, *Aspergillus flavus, Aspergillus niger, Trichoderma harzianum, Penicillium* sp. were isolated from mushroom substrates and identified. Biological activities these fungi were evaluated against three plant pathogens namely against *Fusarium oxysporum, Sclerotium rolfsii, Colletotrichum corchori*. Among the fungal isolates, *Trichoderma harzianum* showed the highest antagonistic activity against all three pathogens, *Penicillium* sp. showed less significant antagonistic activity than *Aspergillus niger* and *Aspergillus flavus. Trichoderma harzianum showed* the highest inhibitory effect in case of *Fusarium oxysporum* (71.75%) followed by *Sclerotium rolfsii* (61.64%) and *Colletotrichum corchori* (57%).

Keywords: Antagonistic effect, Biological efficacy, Pathogenic fungi, Mycelial growth inhibition

INTRODUCTION

Mushrooms, belonging to class Basidiomycotina, are non-green fungal plants occurring seasonally all over the world. Among the 2000 edible species from 10,000 of different types of mushrooms, Oyster mushroom (*Pleurotus ostreatus)* is suitable for the climatic condition of Bangladesh and on the basis of consumption the oyster mushroom is on the second position after button mushroom word widely (Sanchez, 2010). In mushroom cultivation, substrates play the same role as soil executes in plant production (Kwon and Kim, 2004). Sawdust, cotton seed straw, cereal straw, corncob, sugar cane straw, newspaper, waste paper and other plant fibres prevailed in agro and wood industry showed promising result in case of cellulose content (Ragunathan et al., 1996; Kwon and Kim 2004).

The substrates harbour many weed fungi which act as competitor moulds either by competition for food material or through production of toxic substances (Vijay and Sohi, 1987). Isolated *Aspergillus fumigatus, Chetomium thermophile, Mucor pusillus*

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and *T. harzianum* from straw and oyster compost substrates (Wickremasinghe et al., 1999). Different saprophytic and plant pathogenic fungi occurring in the substrate and competing with mushroom mycelium for space and nutrition are *Aspergillus niger, A.flavus, Alternaria alternata, Drechslera bicolor, Fusarium moniliforme, Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Rhizpus stolonifer, Sclerotium rolfsii,, Trichoderma viride* (Sharma et al., 2007; Sharma and Kumar, 2011). Some of these fungi can be used for biological control of different pathogenic fungi like *Sclerotium rolfsii, Colletotrichum* sp. and *Fusarium* spp.

The present study was undertaken to isolate, identify and characterize the fungi associated with selected mushroom substrate and to evaluate their efficacy against three selective pathogenic fungi.

MATERIALS AND METHODS

The experiment was conducted at the Molecular Plant Pathology Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, and Biochemistry laboratory and Mushroom Culture House (MCH) of Biochemistry, Department, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. This study was conducted during the period of January, 2017 to June, 2017.

Isolation of fungi on PDA media

Isolation was done from three kinds of substrates samples (rice straw, saw dust, waste paper). Three contaminated polybags of rice straw was randomly selected from the inoculated polybags. The stock solution was made by taking 1 gm of substrate from each polybag and mixing them with 100 ml sterile distilled water in a conical flask. Then dilution plate technique was followed as described by Dhingra and Sinclair (1985). The same process was also followed in case of saw dust and newspaper poly bags. After finishing the preparation of different dilution, 0.1 ml of 10^{-2} and 10^{-3} dilution was spread over PDA plate previously dried (to remove excess surface moisture) at three replications as described by Goszczynska and Serfontein (1998). The solution was spread with the help of sterilized glass-rod. The inoculated PDA plates were kept in incubation chamber at 25 ± 1^{0}C.The colonies grown over on PDA were recorded after 3-5 days of incubation. Sub cultures were made by transferring a bit of mycelia from the spread plate and transferred on PDA plates using the hyphal tip culture techniques (Tuite, 1969; Mian, 1995) to a new petri dish on the basis of color and morphology of the colony. Identification was done with the help of different books, manuals and publications following the keys suggested by Barnet (1980), Barnett and Hunter (1992), Watanabe (2000), Mathur and Kongsdal (2003), Malone and Musket (1964), Ellis (1971). Purification was carried out by reculturing the fungi by transferring single hyphal tip of each fungus on PDA media and incubated at 22 ± 2^{0}C for 7 days. The contaminated plates were discarded.

Bioassay of isolated fungi by using Dual culture method

Three pathogenic fungi *Fusarium oxysporum (*the causal agent of dry rot of potato), *Sclerotium rolfsii* (the causal agent of foot and root rot of betel vine), *Colletotrichum corchori* (the causal agent of anthracnose of jute) were collected from MS Laboratory Department of Plant Pathology of Sher-e-Bangla Agricultural University, Dhaka. The fungi were cultured in PDA medium. The culture discs cut separately with the help of sterilized cork borer of isolates and pathogen was aseptically transferred and placed at periphery of the petri dish containing the PDA medium. Inoculated with culture discs of the pathogens alone in the petri dish containing PDA served as control. The inoculated petri dishes were transferred into the incubator and incubated at 25^{o}C. The growth of the fungus was observed periodically in petri-plates and measured the mycelial growth (diameter) in each petridishs were taken (Parvin et al., 2016).

After seven days of inoculation (7DAI), radial mycelial growth (mm) of fungus was recorded. The radial mycelial growth (mm) of each fungi (pathogen and antagonist) was measured by taking average of the three diameters taken for each colony (Parvin et al., 2016). Percent inhibition zone was estimated by the following formula as suggested by Johnson and Sekhar (2012).

$$\text{Percentage inhibition of growth} = \frac{C-T}{C} \times 100$$

C = Growth of fungus in control

T = Growth of fungus in treatment

Statistical analysis of data

Data collected during experiment period were tabulated and analyzed following Statistical package MSTAT-C. Treatment means were compared with Duncan's Multiple Range Test (DMRT)

RESULTS AND DISCUSSION

Morphological characteristics of fungal isolates

Aspergillus flavus grew rapidly producing colonies yellow green to green color with white mycelia at the edges (Fig. 1a). The conidia were finely rough, conidia heads were radiate to columnar with loosely packed phialides; the unisertiate conidia heads had radiate vesicle with the philiades covering upto three quarter of the vesicle; while bisertiate the vesicles were spherical to globose (Fig.1b).

Aspergillus niger was fast growing producing black conidial head at the center and white mycelia towards the edge which changed color to brown with age (Fig. 2a). The colonies had thick mat of floccose mycelia beneath the colonies and at the edges. It formed radial furrows very close to each other. Conidia heads were biseriate and

globose in shape with wide spherical to globose vesicle. The stipe was large and wide with smooth and slightly brown color. Conidiophores were smooth walled, hyaline or turning dark towards the vesicle. Conidia was globose to subglobose in shape (Fig 2b). Similar structure was observed by Nyongesa et al., 2015, Wang et al., 1990 and Thilagam et al. 2016.

Trichoderma harzianum produced whitish to greenish colored mycelia in the beginning. Later a deep green color developed in central part and gradually extended to the periphery. Finally, it appeared a whitish green color (Fig. 3a). Mostly globose to subglobose conidia developed on phialides produced in the opposite direction in each point which observed under compound microscope. Conidiophores were septate, hyaline and loosely branched. Main branches produced lateral side branches. All primary and secondary branches arise at 90° angles with respect to the main axis (Fig 3b). Similar features were found by Jahan et al. 2013, Samuels et al. 2002 and Shah et al. 2012.

The mycelial mass of *Penicillium sp* initially appeared white and then gradually turned green with sterile white margin (Fig. 4a). The texture was powdery, mycelia was radially furrowed with heavy sporulation. Hyphae was hyaline and septate. Conidiophores were erect, septate, and branched. Phialides were grouped in brush-like clusters (penicilli) at the ends of the conidiophores (Fig. 4b). Conidia was in long dry chains, round to ovoid, hyaline or greenish, smooth or rough-walled. (Larone, 1995).

Biological efficacy of isolated fungi against three pathogenic fungi

Trichoderma harzianum showed the best performance by reducing the growth of *Sclerotium rolfsii* in duel culture. The inhibition was 61.80%, which was the highest inhibition percentage. *Aspergillus flavus, Aspergillus niger* and *Penicillium* sp. showed inhibition 57.60%, 45.78% and 19.27%, respectively. The present findings agreed with the findings of Bosah et al., (2010). They conducted an experiment on the pure cultures of three antagonistic fungi, *Trichoderma, Penicillium* and *Aspergillus* species against *Sclerotium* sp. Among the three fungal antagonists evaluated for inhibitory efficacy, *Trichoderma* sp. proved to be the most effective as it exhibited the greatest inhibition to *Sclerotium* sp. both at the initial and final tests. This was closely followed by *Aspergillus* sp. with inhibitory effect on the pathogen at. However, *Penicillium* sp. was slightly inhibitory against *Sclerotium* (Table 1, Fig 5).

In *in-vitro* screening, *Trichoderma harzianum* showed best performance by reducing the growth of *Fusarium oxysporum* up to 71.75%, which is the highest inhibition. The lowest result was found in *Penicillium* spp. (28.81%). *Aspergillus niger* and *Aspergillus flavus* showed 49.07% and 33.10%, respectively. The present findings is

partially supported by findings of Kashem et al., (2011). They conducted a series of experiments to assess the effect of 14 isolates of *Trichoderma* spp. *(Trichoderma harzianum* and *T. viride)* for controlling foot and root rot of lentil caused by *Fusarium oxysporum*. The pathogenecity of 12 isolates of *F. oxysporum* and the mass production of an isolate of *T. harzianum* on 25 substrates are also studied. *Trichoderma* isolates inhibited the growth of *F. oxysporum* up to 92.07 % on agar plates (Table 1, Fig 6).

Mycelial growth of *Colletotrichum corchori* in dual culture method was mostly affected by *Trichoderma harzianum* (57.24%) followed by *Aspergillus niger* (47.95%), *Aspergillus flavus* (42.37%) and *Penicillium* sp. (39.96%). The present findings is partially supported by Fitsum et al., 2014. They conducted an experiment on bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is one of the most devastating seed-borne diseases of common bean (*Phaseolus vulgaris L.*) in Ethiopia. Three fungicides viz., Mancozeb, Folpan and Mancolaxyl, and three bioagents viz., *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. Fr. and *Pseudomonas fluorescens* Migula, were screened *in vitro* for their antifungal activities against common bean anthracnose, using the dual culture and microliter double-dilution techniques. The highest percentage of inhibition of the mycelia germination (80.39%) was obtained from T. *viride*, followed by 75.49% from *T. harzianum* and 40.2% from *P. fluorescens* (Table 1, Fig 7).

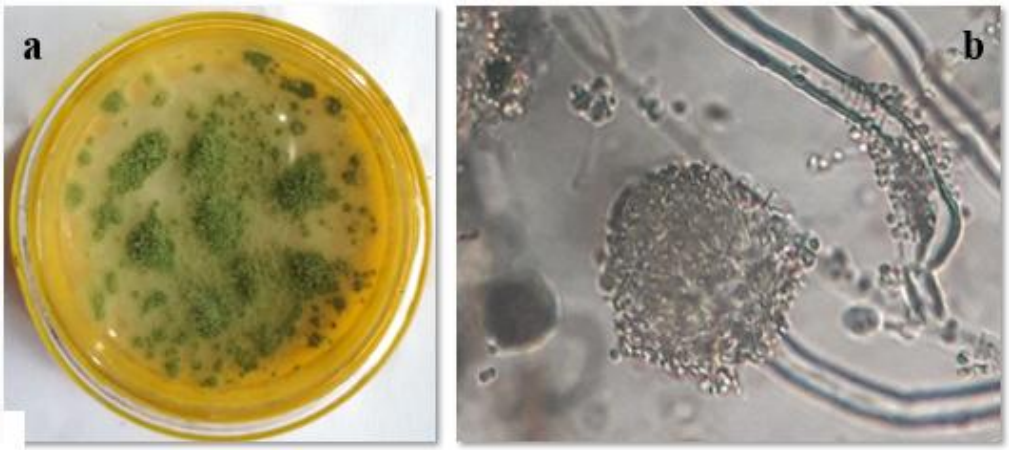

Figure 1. a. *Aspergillus flavus* on PDA medium b. Microscopic appearance of *A. flavus* at 40X

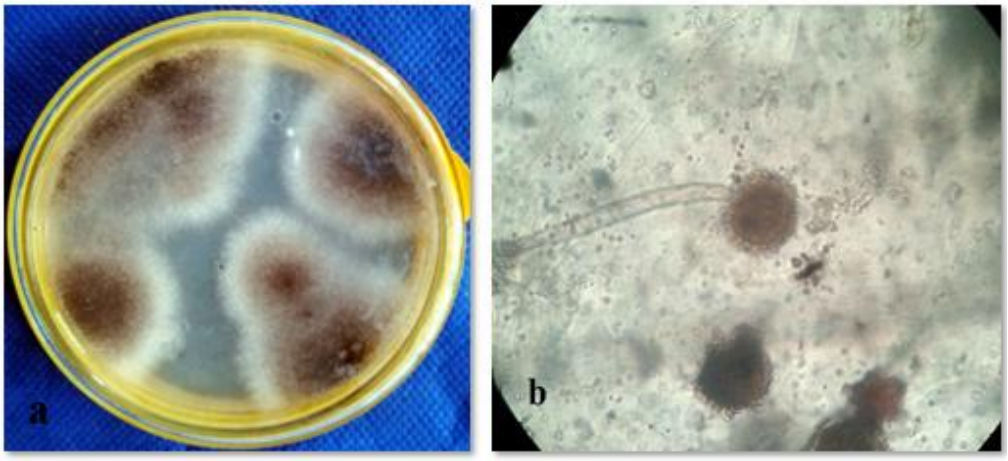

Figure 2. a. *Aspergillus niger* on PDA medium b. Microscopic appearance of *A. niger* at 40X

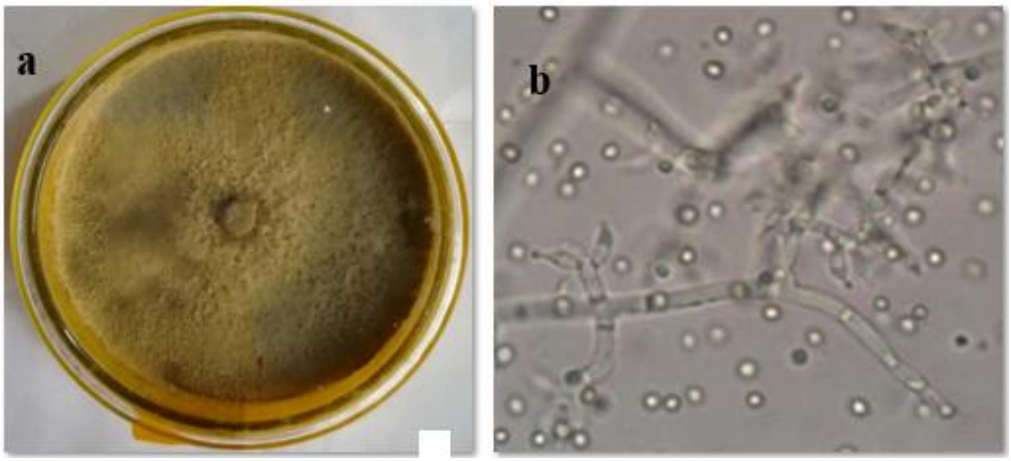

Figure 3. a. *Trichoderma harzianum* on PDA medium b. Microscopic appearance of *T. harzianum* at 40X

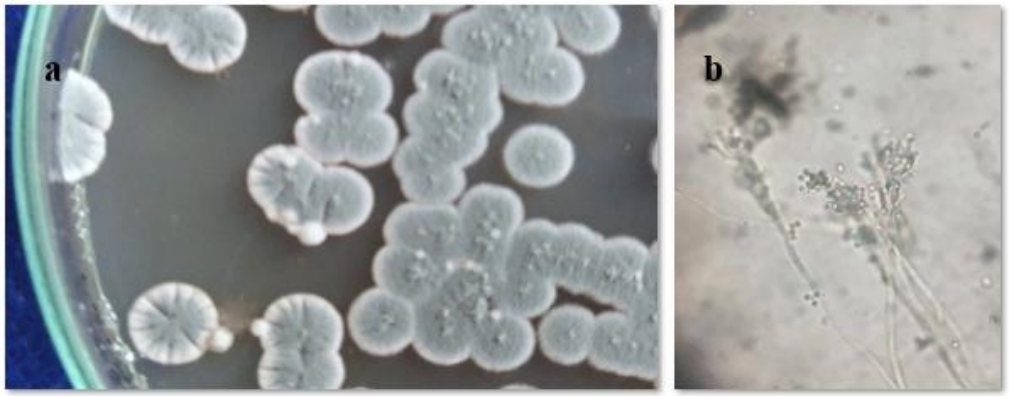

Figure 4. a. *Penicillium* sp on PDA medium, b. Microscopic appearance of *Penicillium* sp at 40X

Table1. Biological efficacy of fungi isolated from mushroom substrate against *Sclerotium rolfsii, Fusarium oxysporum and Colletotrichum corchori*

Treatment	Radial mycelial growth (mm) at 7 DAI*			% Inhibition of mycelial growth at 7 DAI*		
	Sclerotium rolfsii	*Fusarium oxysporum*	*Colletotrichum corchori*	*Sclerotium rolfsii*	*Fusarium oxysporum*	*Colletotrichum corchori*
Penicillium sp.	67.00	30.75	32.30	19.27	28.81	39.96
Aspergillus flavus	35.19	28.90	28.00	57.60	33.10	47.95
Aspergillus niger	45.00	22.00	31.00	45.78	49.07	42.37
Trichoderma harzianum	31.70	12.20	23.00	61.80	71.75	57.24
Control	83.00	43.2	53.8	-	-	-

*In column, DAI = Days after inoculation

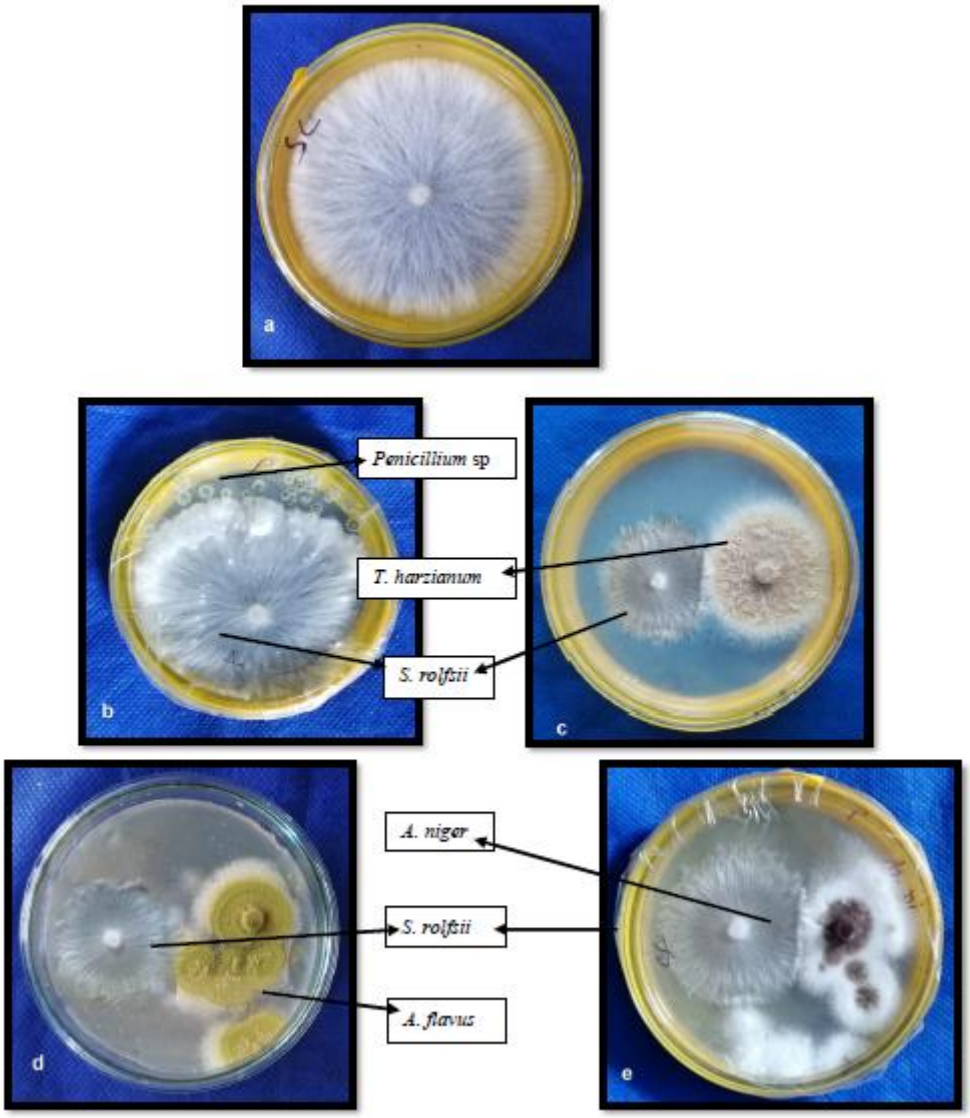

Figure 5. a. Growth of *S. rolfsii* at 7DAI b. Growth of *S. rolfsii* against *Penicillium* sp. at 7DAI c. Growth of *S. rolfsii* against *T. harzianum* at 7DAI d. Growth of *S. rolfsii* against *A. flavus* at 7DA e. Growth of *S. rolfsii* against *A. niger* at 7DAI

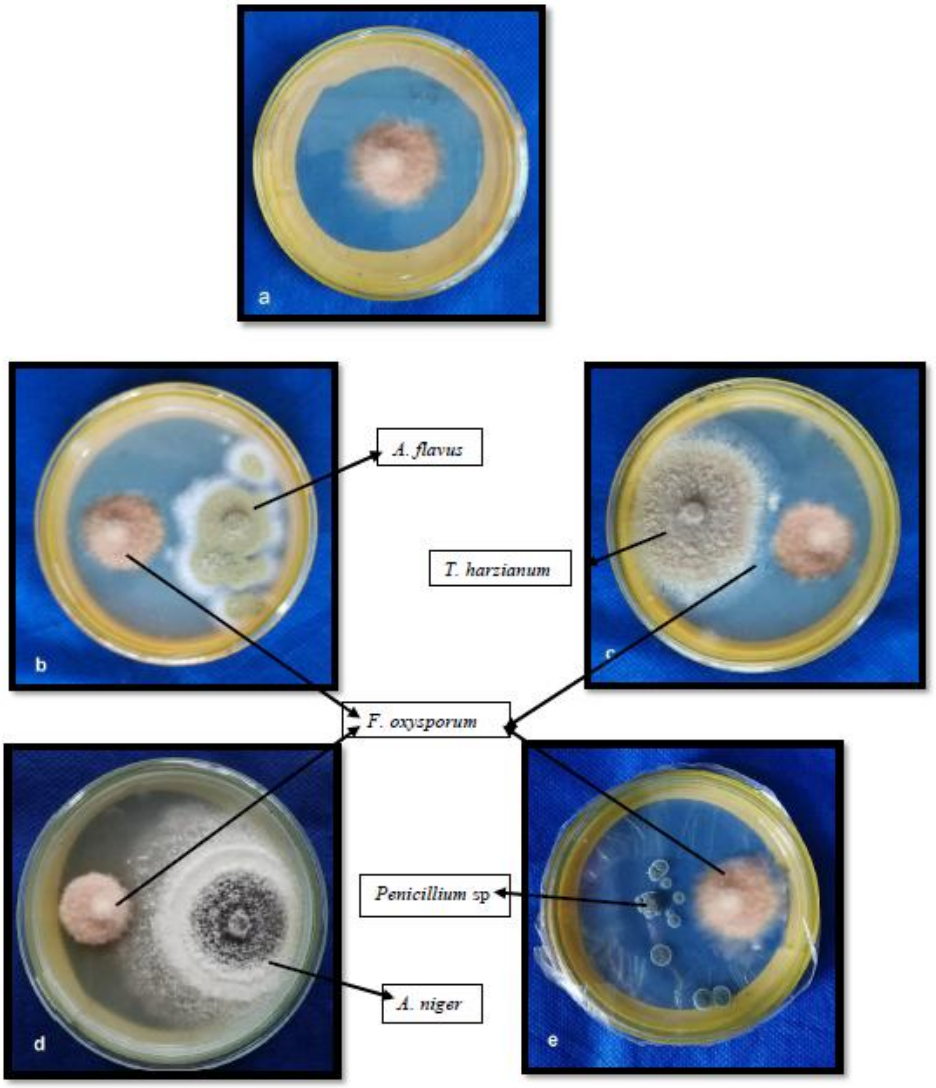

Figure 6. a. Growth of *Fusarium oxysporum* at 7DAI b. Growth of *F. oxysporum* against *Aspergillus flavus* at 7DAI c. Growth of *F. oxysporum* against *T. harzianum* at 7DAI d. Growth of *F. oxysporum* against *Aspergillus niger* at 7DAI e. Growth of *F. oxysporum* against *Penicillium* sp. at 7DAI

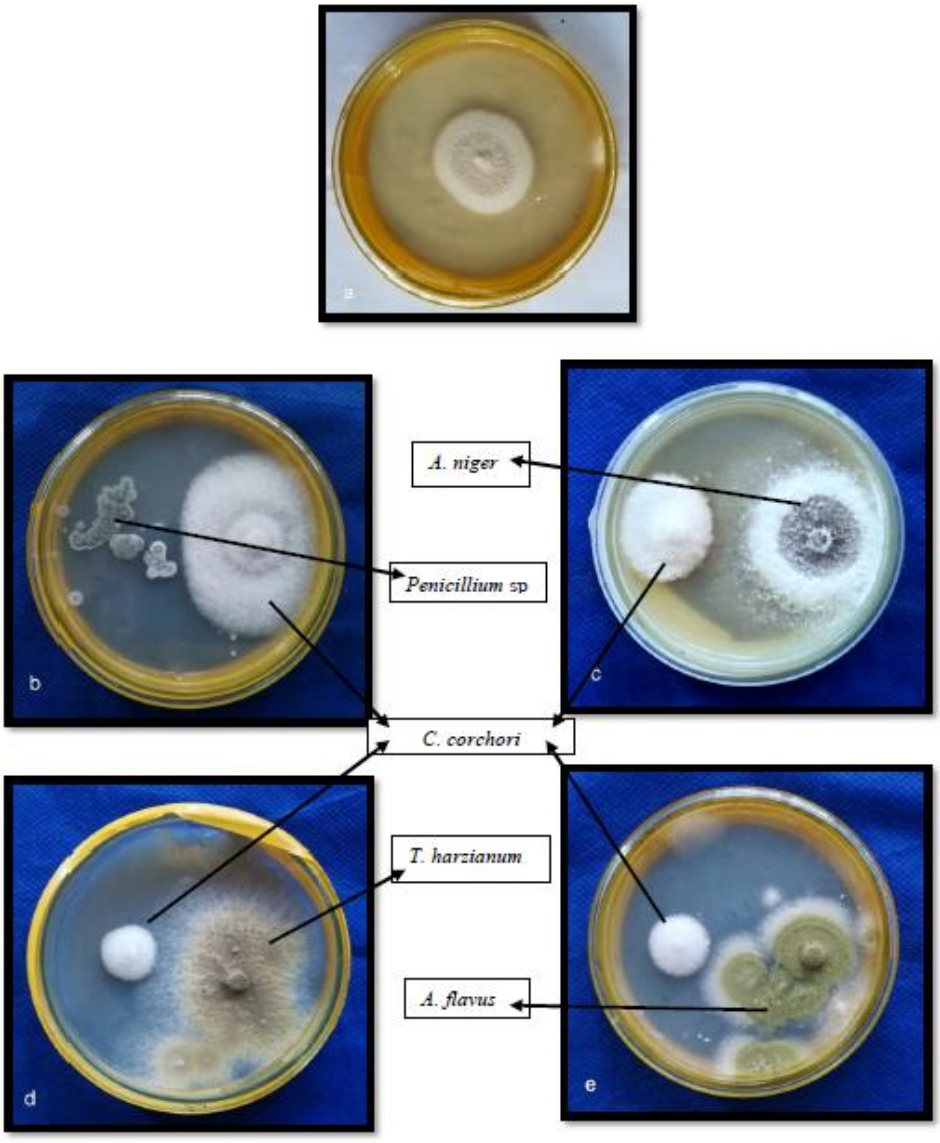

Figure 7. a. Growth of *Colletotrichum corchori* at 7DAI b. Growth of *C. corchori* against *Penicillium* sp. at 7DAI c. Growth of *C. corchori* against *Aspergillus niger* at 7DAI d. Growth of *C. corchori* against *T. harzianum* at 7DAI e. Growth of *C. corchori* against *Aspergillus flavus* at 7DAI

CONCLUSION

Biological efficacy of fungal isolated from mushroom substrates against three pathogenic fungi were studied and found significant variation in terms of percent inhibition of radial mycelial growth of pathogenic fungi. In case of *Fusarium oxysporum* the highest inhibition was observed against *T. harzianum* (71.75%) and the lowest against *Penicillium* sp (28.58%). In case of *Sclerotium rolfsii* highest inhibition observed against *T. harzianum* (61.64%) and the lowest against *Penicillium* sp (19.55%). In case of *Colletotrichum corchori* highest inhibition observed against *T. harzianum* (57%) and the lowest against *Penicillium* sp (39.59%). It has been observed that among the fungal antagonist used against pathogenic fungi the most effective was *T. harzianum.*

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