Biolife 2(3):918-923
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

SCREENING OF ANTIFUNGAL ACTIVITY OF *PLEUROTUS OSTREATUS* AND *AGARICUS BISPORUS*

Vinay Kumar[1]* and Udit Yadav[2]

[1-2] *Microbiology lab, Department of Plant Sciences, M.J.P. Rohilkhand University, Bareilly-243006(Uttar Pardesh), India*

E-mail: vinaypbtbhu@gmail.com

ABSTRACT

The fruiting body powder of *Pleurotus ostreatus* (oyster mushroom) and *Agaricus bisporus* (button mushroom) was extracted with ethanol by using the agar well diffusion technique, the extract were tested for their antifungal activity against six fungi belonging to 5 genera viz. *Aspergillus flavus, Aspergillus fumigatous, Penicillium chrysogenum, Sporotricum carnis, Humicola grisea and Thermoascus aurantiacus*. The extract of *Pleurotus ostreatus* showed the maximum inhibition against *Penicillium chrysogenum* and minimum against *Aspergillus flavus*. The extract of *Agaricus bisporus* showed the maximum inhibition against *Humicola grisea* and minimum against same fungi *Aspergillus flavus.*

Key Words:- Antifungal activity, Fruiting Body, *Pleurotus ostreatus* , *Agaricus Bisporus*, ethanol extract.

INTRODUCTION

Mushroom is defined as "A macro fungus with a distinctive fruiting body which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand (S.G. Jonathan et.al, 2003). Mushrooms are rich in carbohydrate and protein. Proteins of these fungi have been reported to show several biological activities like antiproliferative, immunomodulatory, anti-viral, antifungal and anti bacterial (A.Turkoglu et. Al., 2006, H.X. Wang et. al., 2000, H.X.Wang et.al., 2004, M.H. Solak et.al.,2006, R. Cohen et.al.,2002, S. Bender et.al.,2001). Culture extracts of *Agaricus* sp. of inhibitory action on the gram+ and gram- bacterial growth was achieved (S. A. Waksman et.al., 1922).

Agaricus bisporus, an edible mushroom with its enriched protein can have antibacterial properties against some important bacterial pathogens. Fruiting body and the mycelium of mushrooms contain compounds giving antimicrobial activity. There are many report the effectivity of different mushroom extracts against several microorganisms (A. Demirhan et.al., 2007,] A.Turkoglu et.al., 2006, J.C. Gilman et.al., 2001, Kiran Nehra et.al., 2012, L. Barros et.al., 2007, P.H.K. Ngai et.al.,2004).

But, although a number of mushroom varieties with antimicrobial activities have been identified, a greater number still remain unidentified.

Mushrooms contain some potential antibacterial and antifungal compounds such as peptide eryngin and polypeptide alveolarin originated from *Pleurotus eryngii* and *Polyporus alveolaris*, respectively which have highly antifungal potential (H.X.Wang et.al., 2004).

Keeping in view the medicinal importance of mushrooms in our daily life and the limited reports on the detailed studies of *Pleurotus ostreatus* and *Agricus Bisporus*, the present investigation has been undertaken to explore *in-vitro* antifungal activity of fruiting body Ethanolic extracts of *Pleurotus ostreatus* and *Agricus bisporus.* The result can emphasize the usefulness of the mushrooms as a natural source of antifungal agents and may open a window to find alternative compounds to substitute the current antifungal products which are being ineffective by the fungal resistance.

MATERIALS AND METHODS

Study site and location:

Bareilly is a prominent city in northern Indian state of Uttar Pradesh. Standing on the Ramganga River, it is the capital of the Bareilly division and the geographical region Rohilkhand. It is located 252 kilometers (157 mi) north of the state capital, Lucknow and 250 kilometers (155 mi) east of the national capital new Delhi., Bareilly is located at 28°10′N 78°23′E and lies in northern India. Bareilly is known to have moderate climate. The city lies entirely in the Ganges plains. The low-lying Ganges plains provide fertile alluvial soil suitable for agriculture. Nature of the soil is generally sandy loam, loam, clay loam, silt loam.

Method for collection of soil samples:

The soil samples were collected from three different fields in various locations of Bareilly city. Soil samples of three different fields at Bareilly city were collected during December 2012 to January 2013. The soil samples were collected from different fields (Table: 1) (up to 15cm depth) into a small sterilized polythene bags and brought to laboratory for further studies.

Isolation of fungi from the soil samples:

The soil micro fungi were enumerated by modified Soil Dilution method (S. A. Waksman et.al., 1922) on yeast powder starch soluble Agar (YpSs) 1gram of soil sample was suspended in 10 ml of double distilled water to make microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi. 1 ml of microbial suspension or solution of each concentration were added to sterile Petri dishes (triplicate of each dilution) containing 15 ml of sterile yeast powder starch soluble Agar (Ypss). One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth. The Petri dishes were then incubated at 28±20 C in incubator. The plates were observed everyday up to three days.

Identification of the soil fungi:

Fungal morphology was studied microscopically by observing colony features (Color and Texture) and microscopically by staining with lacto-phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores (K.R. Aneja et.al., 2001). The fungi were identified with the help of literature (A. Nagamani et.al., 2006, J.C. Gilman et.al., 2001).

Table 1: soil samples collected from different places in Bareilly city

Sample No.	Place
1.	C.B. Ganj
2.	Karamchari Nagar
3.	Chaudhary Talav

Collection of *Agricus bisporus* & Cultivation of *Pleurotus ostreatus:*

A. The mushroom, *Agricus bisporus* used in this study was obtained from Krishi Vigyan Kendra, Post-Ujwa, Via- Nazafgarh, New Delhi, India.

B. To Cultivate *Pleurotus ostreatus* used in this study and mother spawn was obtained from agricultural technology and information

center (ATIC), G.B. pant Agricultural and technology University, Pantnagar, India.

Cultivation & harvesting of *Pleurotus ostreatus*:

Substrate used for the cultivation of *Pleurotus ostreatus* was wheat straw. The substrate was soaked in water for overnight, There after straw was taken out from the tank and put into another tank with hot water for 1-2 hrs. There after straw was taken out from the tank and spread on clean cemented floor treated with 2% formalin for two hours and then spawning is done and harvesting was started after 18 to 20 days (E. H. Amuneke et.al., 2011).

Developing Fruiting

Developed Fruiting

Preparation of mushroom extracts:

The fruiting bodies of *Agricus bisporus* and *Pleurotus ostreatus* were washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles; thereafter, they were allowed to dry in an oven at 40^{o}C. The dried mushrooms were grounded to a fine powder and stored in airtight containers until used for further studies. To 100g of each i.e. *Agricus bisporus* and *Pleurotus ostreatus* (fruiting body powder), 100 ml of ethanol solvent was added for preparing extracts. Extraction was done on rotatary shaker for 48h at room temperature. After filtering through filter paper (Whatmann No.1), the supernatant in different solvents was recovered. These extracts were stored at 4 ^{0}C until used for evaluating the antifungal activity (Kiran Nehraet.al., 2012).

EXPERIMENTAL RESULTS

Isolation of Fungus:

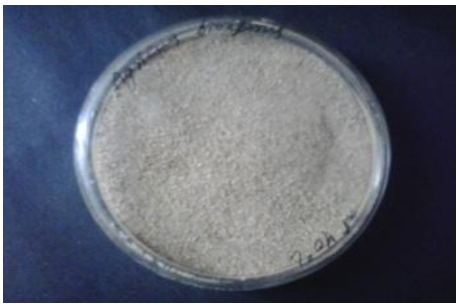
During the investigation period 82 fungal colonies of 6 fungal species were observed (Table-2). The maximum fungal species belongs to *Basidiomacetes* were observed. Among the isolates the genera *Aspergillus* were dominant (Table-2). The soil mycoflora in different fields viz; C.B. Ganj, Karamchari Nagar and Chaudhary Talav were observed. The most common among them viz; *Aspergillus fumigatous* (14.6%), *Aspergillus flavus* (10.9%), *Humicola grisea* (10.9), *Penicillium chrysogenum* (9.7%), *Thermoascus aurantiacus*(8.5%) *and Sporotricum carnis* (7.3%) were isolated and characterized.

Table 2: Frequency of mycoflora in different collection sites of Bareilly city

S. NO.	Site	Average No. of total colonies	Average no. of individual colonies					
			Aspergillus		*Penicillium chrysogenum*	*Sporotricum carnis*	*Humicola grisea*	*Thermoascus aurantiacus*
			Afl	Afu	Pc	Sc	Hg	Ta
1	C.B. Ganj	28	3	4	3	2	2	2
2	Karmcharinagar	31	4	5	2	4	3	3
3	Chaudhary Talav	23	2	3	3	-	4	2
Total		82	9	12	8	6	9	7
% Contribution			10.9	14.6	9.7	7.3	10.9	8.5

Afl- *Aspergillus flavus ;* Sc- *Sporotricum carnis;* Afu- *Aspergillus fumigates;* Pc- *Penicillium Chrysogenum;* Ta- *Thermoascus aurantiacus;* Hg- *Humicola grisea*

Powder of *Pleurotus ostreatus*



Powder of *Agricus bisporus*

Ethanolic Extract

Antimicrobial activity:

The results of the antifungal response shown by the ethanol extracts of *Agricus bisporus* and Pleurotus ostreatus are summarized in figures 1 to 4. Ethanolic extracts prepared exhibited variable degree of antifungal activity against the tested fungus such as *Aspergillus flavus, Aspergillus fumigatous, Penicillium chrysogenum, Sporotricum carnis, Humicola grisea and Thermoascus aurantiacus*. However, the figure indicates that the extracts prepared in ethanol solvents consistently displayed better antifungal activity. The Ethanolic extract of *Agaricus Bisporus* and *Pleurotus ostreatus* exhibited different levels of antifungal activity against six fungus out of three fungus do not show inhibition. The maximum significant inhibition activity of *Agaricus Bisporus* was observed with *Humicola grisea* and minimum against *Aspergillus flavus*. The maximum significant inhibition activity of *Pleurotus ostreatus* was observed with *Penicillium chrysogenum* and minimum against *Aspergillus flavus*. *Agaricus Bisporus* and *Pleurotus ostreatus* were not show antifungal activity against *Aspergillus fumigatous, Sporotricum carnis and Thermoascus aurantiacus.*

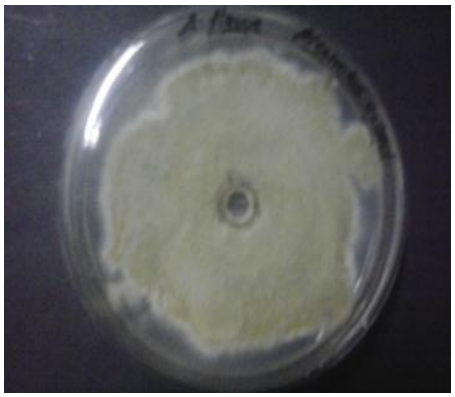
Figure: Isolation of different fungus on yeast powder starch soluble Agar (YpSs) media

Aspergillus fumigatous *Thermoascus auranticus*

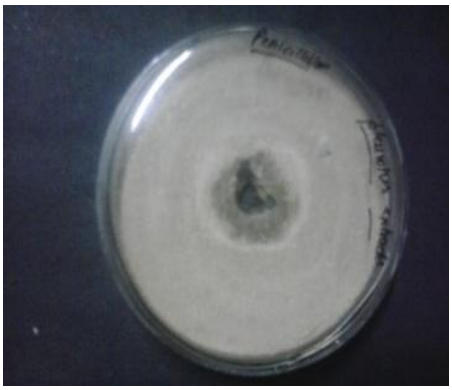
Penicillium chrysogenum *Sporotricum carnis*

Humicola grisea *Aspergillus Flavus*

Figure: Antifungal activity of Ethanolic extract of *Pleurotus ostreatus* and *Agaricus Bisporus*



(1) *Ple. Ostr.* against *Asp. Fla.*



(2) *Ple. Ostr*. against *Peni. Chry.*

(3) *Aga. Bis.* against *Asp. Fla.*

(4) *Aga. Bis.*against *Humi. Gri.*

CONCLUSION

Comparative study of the most common among them viz; *Aspergillus fumigatus* (14.6%), *Aspergillus flavus* (10.9%), Humicola grisea (10.9), *Penicillium chrysogenum* (9.7%), Thermoascus aurantiacus (8.5%) *and Sporotricum carnis* (7.3%) were isolated and characterized. The Ethanolic extract of *Pleurotus ostreatus* showed the maximum inhibition against *Penicillium chrysogenum* and minimum against *Aspergillus flavus*. The extract of *Agaricus bisporus* showed the maximum inhibition against *Humicola grisea* and minimum against same fungi *Aspergillus flavus*.

ACKNOWLEDGEMENTS

The authors are grateful to the department of Plant Science of M.J.P. Rohilkhand University to for beneficial discussions and experimental equipment support for the present study.

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DOI: https://dx.doi.org/10.5281/zenodo.7224733
Received: 11 July 2014;
Accepted; 23 August 2014;
Available online : 12 September 2014