

Original Research Article

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Screening of Antifungal Activity of *Pleurotus pulmonarius*, *Pleurotus florida* and *Shizophyllum commune*

Vinita Dahima¹, A. Doshi¹ and Hanuman Singh^{2*}

¹Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap
University of Agriculture and Technology, Udaipur 313 001, Rajasthan, India

²College of Horticulture & Forestry, Agriculture University, Kota, 324001, Rajasthan, India

*Corresponding author

ABSTRACT

Antifungal activities of the three mushrooms *Pleurotus pulmonarius*, *Pleurotus florida* and *Schizophyllum commune* ethanol extracts were evaluated against some plant pathogenic fungi (*Aspergillus flavus*, *Aspergillus niger* and *Alternaria solani*, *Colletotrichum graminicola*, *Fusarium oxysporum*) by using agar well diffusion method. Minimum inhibitory concentration (MIC) was investigated for 1-20 mg/ml concentration to find out the lowest concentration of the sample that inhibits the growth of the test organisms. The standard agar dilution protocol with doubling dilution was used. The result revealed that the three mushrooms extract used in the present investigation possessed varying degrees of antifungal activities against the test plant pathogenic fungi. Ethanolic extract of *Pleurotus florida* showed highest inhibition activity against *Fusarium oxysporu*, and *S. commune* showed maximum zone of inhibition (ZOI) against *A. niger*, while, *P. pulmonarius* showed maximum zone of inhibition (ZOI) against *A. solani*. The minimum inhibitory concentrations (MIC) ranged between 9.67 and 18.98 mg/ml for fungi, the lowest MIC, the extract was still be effective because of the presence of bio-active compounds. The findings revealed that the mushrooms extracts tested in the current work contains potential therapeutic compounds against some of the economically important plant diseases caused by bacteria and fungi.

Keywords

Antifungal,
Mushrooms,
*Fusarium
oxysporum*,
Minimum inhibitory
concentrations,
Agar well diffusion

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Introduction

Although, fungicides and antibiotics have been very effective in controlling the fungal and the bacterial diseases, respectively but the use of those chemicals leads to health and environment hazards. Despite the use of half a million tones fungicides and pesticides annually, one third of all crop production is

still lost. Continued use of fungicides is threatening the environment and health and is responsible for some major problems. Firstly, some fungi have acquired resistance against fungicides particularly the systemic fungicides; secondly, some fungicides are not biodegradable and tend to persist for years in the environment. Therefore, human health and environmental safety are the two most

important issues in the long term application of pesticides, fungicides and antibiotics (Lin, 1995). Due to increasing awareness about the risk involved in use of chemicals much attention is being focused on alternative methods of pathogen control. The increasing failure of chemo-therapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the screening of novel sources for their potential antibacterial and antifungal activity.

In response to such aggravated problems, researchers are now focused on new effective biological pesticides with low toxicity wild and under-utilized mushrooms species are identified as a potential resource in this regard. It is known that, macro fungi need antibacterial and antifungal compounds to survive in their natural environment.

Medicinal mushrooms are able to synthesize a great amount of secondary metabolites that present antitumoral, antiviral, anti-inflammatory, antibacterial, antifungal and anti-yeast activities. The antifungal properties of mushrooms also provide an additional advantage by lowering food safety risks associated with fungicide use in cultivated mushrooms, as mushrooms with antifungal property can suppress undesirable fungi, such as., *Aspergillus spp.* and *Penicillium spp.* Such bioactive molecules reported to be present in both edible and non-edible mushroom species (Quang *et al.*, 2006).

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 (Wang *et al.*, 2004). keeping in view the importance of mushrooms in having potential antimicrobial properties the present research work has been proposed to evaluate the antimicrobial potentials of ethanol extracts of three

mushrooms against plant pathogenic fungi.

Materials and Methods

Fungal cultures

Alternaria solani, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum graminicola* and *Fusarium oxysporum*, obtained from the Department of Plant Pathology – RCA were used.

Fruiting bodies

Three mushroom species fruiting bodies; *Pleurotus pulmonarius*, *Pleurotus florida* and *Schizophyllum commune* were obtained from AICRP (Mushroom). These species were collected from different forest areas of Rajasthan (Northwest of India) in the rainy season of 2014 by Dr. Anila Doshi. The basidiomycetes were identified by their sporeprints, and comparing their morphological, anatomical and physiological characteristics with the standard descriptions of Zoberi (1978), and that of Alexopolous *et al.*, (1996) (Fig. 1)

Preparation of crude ethanol extract

The fruit bodies of three mushrooms viz: *Pleurotus pulmonarius*, *Pleurotus florida* and *Schizophyllum commune* species were cut into pieces and dried in an oven at 40°C. The dried carpophore was pulverized in amoulinex blender. Ten g of each powder carpophore was soaked separately in 100 ml of 95% ethanol in an Erlenmeyer flask. The flasks were covered with aluminium foil and kept at 25°C for 7 days. After 7 days, the content of the flask was filtered with Whatman filter paper No. 1. The filtrate obtained was concentrated in a rotary evaporator at 40°C. The dried extract was stored in a refrigerator at -4 °C for further analysis (Jonathan and Fasidi, 2003).

Screening of antifungal activity of mushroom sample

Preparation of samples

To prepare sample for antibacterial assay 200 mg of the each extract was dissolved in 10 ml of DMSO to get 20 mg/ml concentration. This stock solution was sterilized by filtration through a 0.2 μm membrane filter (Ali-Shtayeh *et al.*, 1998; Tepe *et al.*, 2005). Pure DMSO was used as negative control Standard antibiotics;

Agar well method

The plant pathogenic fungal cultures (*Alternaria solani*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum graminicola*, *Fusarium oxysporum*) were incubated at room temperature for 48 hrs in Potato Dextrose broth media. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 Mc Farland turbidity standard tubes. Potato Dextrose agar media (20 ml) were poured into sterilized Petri dishes (10 x 90 mm diameter) after inoculation with the fungal cultures (100 μl) and distributed homogeneously and allowed to solidify. With the help of sterilized cork borer a well of 6 mm in diameter was bored at the centre of the media in the plate. The mushrooms extracts of 100 μl was filled into the wells of agar plates directly. The plates were incubated at $28 \pm 1^\circ\text{C}$ for 3-4 days. The pre test result of mancozeb – 1000 ppm against fungal pathogens in current studied work showed that, it has been controlled effectively and hence, mancozeb was selected as reference. After the incubation period, the inhibition zone (diameter) formed on each media were measured. Inhibitory activity of the DMSO was also tested as negative control. Studies were performed in fourth time and the results were expressed as average values.

Determination of minimum inhibitory concentration (MIC)

The MIC study was aimed to find out the lowest concentration of the sample that inhibits the growth of the test organisms. The standard agar dilution protocol with doubling dilution was used. The MICs of the extract for each test microorganism were regarded as the agar plate with the lowest concentrations without growth (Oboh *et al.*, 2007 and Vamanu, 2012). The standard concentration of the extract used was 20.0 mg/ml. Dimethylsulfoxide (DMSO) was used as the diluent. Mushrooms with activities at this concentration were regarded as having antimicrobial properties while other with no activity at this concentration was disregarded (Hirasawa *et al.*, 1999).

Statistical analysis

All the data recorded from series of experiments was subjected to analysis of variance (ANOVA) by using appropriate statistical tools and techniques.

Results and Discussion

In the current study, different crude extracts of mushrooms *Pleurotus pulmonarius*, *Pleurotus florida* and *Schizophyllum commune* have been used *in vitro* to evaluate the inhibitory effects against plant pathogenic fungi. Standard concentration (20.0mg/ml) was prepared for ethanolic extracts of these species.

The ethanol extract of all the three higher fungi showed varying degrees of antifungal properties against the test fungi (Table 1). This is because the three mushroom having active ingredients, produced different metabolites and antifungal peptide, which act as bioherbicides, bioinsecticide and biofungicides product which inhibited

mycelial growth of pathogenic fungi (Wang and Ng 2004; Gregori *et al.*, 2007; Badalyan *et al.*, 2002; Bennett *et al.*, 2001 and Teoh *et al.*, 2012). *Schizophyllum commune* showed maximum zone of inhibition (32.56 mm) against *Aspergillus niger* (Plate 1) which was followed by inhibition zone of 28.56 and 25.34 mm in diameter against *Colletotrichum graminicola* and *Alternaria solani* respectively (Table 1).

Moreover, *Fusarium oxysporum* highly susceptible to extract of *P.florida* at 9.67mg/ml (Plate 2, Table 2). *Pleurotus pulmonarius* produced the largest zone of inhibition (30.67 mm) against *Alternaria solani* (Plate 3) followed by *Fusarium oxysporum* and *Aspergillus flavus*, with 30.50 and 28.35 mm zone of inhibition, respectively. Inhibition zone of 26.78 and 25.67 mm were appeared against *Aspergillus niger* and *Colletotrichum graminicola*, respectively, when the crude extracts from the *P. pulmonarius* mushroom were used. The minimum inhibitory concentrations (MIC) ranged between 9.67 to 18.98mg/ml for

bacteria lowest MIC, the extract was still be effective because of the presence of bio-active compounds. The MIC of *P. pulmonarius* was found to be higher for *Colletotrichum graminicola* at 18.98 mg/ml while, *Fusarium oxysporum* had lowest MIC of *P. florida* extract at 9.67mg/ml (Table 2).

The results of the present investigation revealed that ethanolic extract of *Pleurotus florida* showed highest inhibition activity against *Fusarium oxysporum* with lowest MIC. *In vitro* antagonistic activity of *P. florida* to *Fusarium spp* has been reported by Chu *et al.*, 2005, Ngai and Ng 2004, Hassan *et al.*, 2011). The extract was still, be effective because of the presence compound such as of bioactive chitinase, protease and phenol compounds that can inhibit the growth of microorganism.(Hassan *et al.*, 2011). Among the three mushrooms *S. commune* showed maximum zone of inhibition against *A. Niger*. 14 days-old culture filtrates of *S. commune* exhibited 90% inhibition of *A. niger* (Balaji, 2009). The observed results were highly significant at 5% level of significance.

Table.1 Size of inhibition zone (mm) by ehanolic extracts of mushroom against the sample of plant pathogenic fungi (well method)

Plant Pathogenic Fungi	Average size of Inhibition Zone (mm)			
	<i>Schizophyllum commune</i>	<i>Pleurotus florida</i>	<i>Pleurotus pulmonarius</i>	Mancozeb (1000 ppm)
<i>Alternaria solani</i>	25.34	27.67	30.67	59.89
<i>Aspergillus niger</i>	32.56	20.78	26.78	70.53
<i>Aspergillus flavus</i>	20.67	14.85	28.35	64.67
<i>Colletotrichum graminicola</i>	28.56	20.89	25.67	64.27
<i>Fusarium oxysporum</i>	25.26	35.56	30.50	79.89
SEm±	0.671	0.732	0.960	1.922
CD at 5%	1.92**	2.10**	2.76**	5.52**
CV%	4.39	5.30	5.86	4.91

Table.2 Minimum inhibitory concentrations (MIC) of the ethanol extracts of the three mushroom against the plant pathogenic fungi

Test Fungi	MIC (mg/ml)		
	<i>Schizophyllum commune</i>	<i>Pleurotus florida</i>	<i>Pleurotus pulmonarius</i>
<i>Alternaria solani</i>	15.75	11.90	12.78
<i>Aspergillus niger</i>	17.38	15.90	10.89
<i>Aspergillus flavus</i>	12.89	13.89	14.50
<i>Colletotrichum graminicola</i>	10.89	11.89	18.98
<i>Fusarium oxysporum</i>	14.67	9.67	17.89
SEm±	0.40	0.34	0.35
CD at 5%	01.17**	00.99**	01.01**
CV%	04.94	04.75	04.08



Fig.1 Fruiting body and extraction product of (a) *Schizophyllum commune*, (b) *Pleurotus pulmonarius*, (c) *Pleurotus florida*

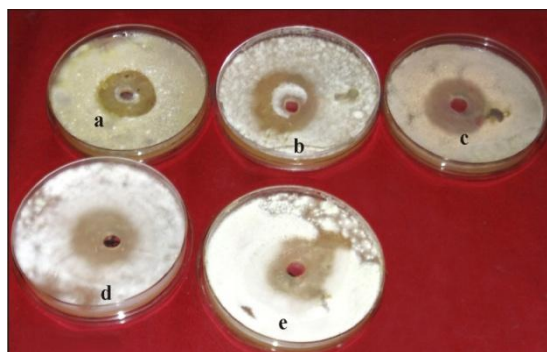


Plate.1 Size of inhibition zone (mm) by *Schizophyllum commune* against the sample of plant pathogenic fungi (Well method) a. *Alternaria solani* b. *Colletotrichum graminicola*; c. *Aspergillus niger*; d. *Fusarium oxysporum*; e. *Aspergillus flavus*

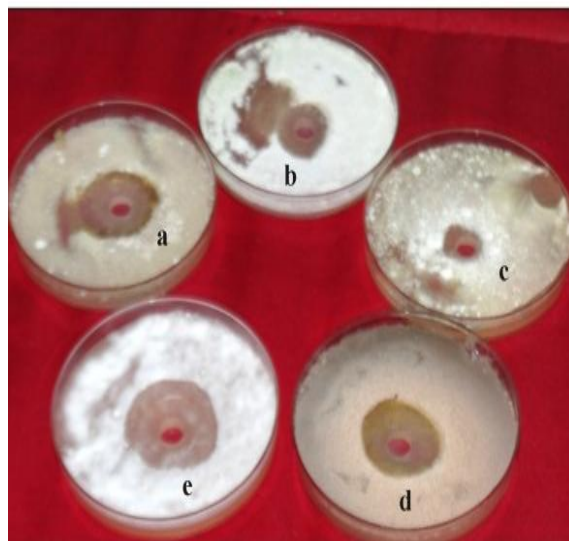


Plate.2 Size of inhibition zone (mm) by *Pleurotus florida* against the sample of plant pathogenic fungi (Well method) a. *Alternaria solani* b. *Colletotricum graminicola*; c. *Aspergillus flavus* . d. *Aspergillus niger*; e. *Fusarium oxysporum*;

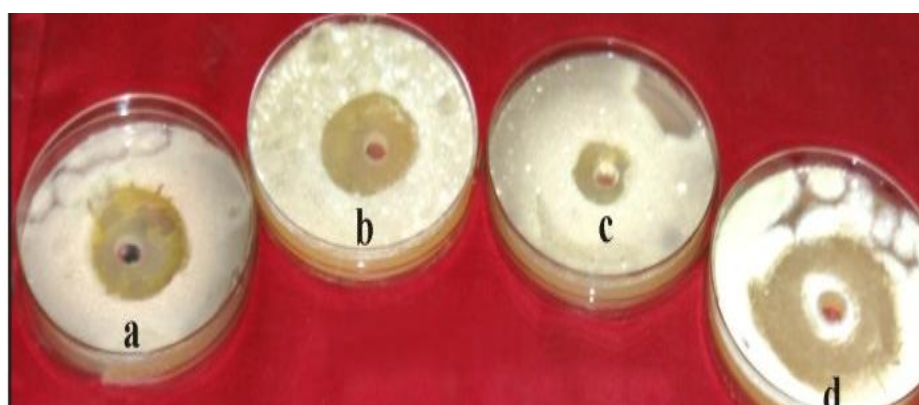


Plate.3 Size of inhibition zone (mm) by *Pleurotus pulmonarius* against the sample of plant pathogenic fungi (Well method) a. *Aspergillus niger*; b. *Colletotricum graminicola*; c. *Alternaria solani*; d. *Aspergillus flavus*;

The antibacterial activity was observed in *Pleurotus pulmonarius* against plant pathogenic fungi. The nature of inhibition indicates possible presence of polysaccharides from *P. pulmonarius* (Wasonga *et al.*, 2008) do not directly act but altered the immune response of host by increasing the macrophage activity which then kill the pathogens (Wasser and Weis 1999).

This observation can be explained by different active compounds were extracted with different mushrooms and thus resulted in

different antimicrobial activity. It also believed that the variation of antimicrobial activity of mushrooms reflects the genetic differences of the species at the intraspecific level and variety of factors like the nature of environment, growth media, mushroom species solvent system for extraction, and obviously the genetic structure of mushroom specie. On the basis of the experimental evidence, this study showed that extracts of three mushrooms had curative properties against fungi infections and thus they can be used as potential antimicrobial agents. further

studies on isolation and screening of the active compounds might deliver a better source for emerging new therapeutic agents and therefore find its application in nutraceuticals and pharmaceuticals industries.

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