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Original Research Article

Antifungal Activity of Accumulated Fruit Body Extract of *Lentinus squarrosulus* and *Pleurotus pulmonarius* against Plant Fungal Species

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A B S T R A C T

In our study, we taken two fruit body extract of the mushroom species *viz. Lentinus squarrosulus* and *Pleurotus pulmonarius* are showed significant antibacterial activity. In our In vitro study, we taken 10 treatments (with Control without heavy metals and Control). $PbSO_4$ & $CdSO_4$ with *Pleurotus pulmonarius* were found maximum effective in control of fungal species *Colletotrichum graminicola*, *Alternaria solani* and *Fusarium oxysporium* Moreover, $CuSO_4$ & $ZnSO_4$ were found moderately effective with *Lentinus squarrosulus* against *Colletotrichum*, $CdSO_4$ & $ZnSO_4$ were found moderately effective with *Lentinus squarrosulus* against *Alternaria* and $PbSO_4$ & $CdSO_4$ were found moderately effective with *Lentinus squarrosulus* against *Fusarism oxysporium*. It shows that antibacterial property of these extracts should be due to two various reasons. First, inherent compounds present in fruit bodies and second, accumulated concentration of heavy metals from substrate. Combined effect of both reasons was stronger than individual effects. Both mushroom species alone (without heavy metals) presented weak antibacterial activity against all three bacterial species.

Keywords

Mushroom, Antibacterial, Extract, Fruit body, fungus

Introduction

Basidiomycetes produce a large number of secondary metabolites (Anke, 1989) which showed antibacterial, antifungal, antiviral, cytotoxic and hallucinogenic activity or which can be source of plant growth regulators on flavour's (Janssens *et al.,* 1992; Breheret *et al.,* 1997; Marumoto *et al.,* 1997; Gupta *et al.,* 2018 and Dahima *et al.,* 2020). Mushrooms are rich source of natural antibiotics, among them, the cell wall glucans are well known for their immunomodulatory properties, and many of the externalized secondary metabolites (extra cellular secretion by mycelium) combat bacteria and viruses. Also, it is reported that antimicrobial compounds could be isolated from many mushroom spp. Bioactive molecules have been isolated from edible and non-edible spp. (Quang *et al.,* 2006). Wasser and Weis (1999) and Lindequist *et al.,* (2005) reported

bioactive properties mushroom including antibacterial, antifungal, antioxidant, antiviral, antitumor cytostatic, immune suppressive, antiallergic, and antiatherogenic activities. Dahima *et al.,* (2020) screening of antifungal activity of *P. pulmonarius*, *P. Florida* and *Shizophyllumcommane*. Determination of antimicrobial activity profile of all isolates tested against pathogenic bacteria and fungi indicated that the concentrations of bioactive components directly influence the antimicrobial capability.

Materials and Methods

Fungal Cultures

Alternaria solani, Colletotrichum graminicola and *Fusarium oxysporium* obtained from the Department of Plant Pathology, RCA, Udaipur were used. The stock culture of these organisms had already been identified and typed. Fungi were grown aerobically at 30^{o} C on the liquid PDA and antifungal testing was carried out on the PDA plates.

Mushroom Samples

Heavy metal accumulated fruit body of *Pleurotus pulmonarius* and *Lentinus squarrosulus.*

Extract Preparation

The extraction of the heavy metal accumulated mushrooms *Pleurotus pulmonarius* and *Lentinus squarrosulus* carried out using ethanol solvents. For ethanol extraction, 10gm of the pulverized sporophore was separately soaked in 100 ml 95 percent ethanol inside 150 ml conical flasks. These were covered with aluminium foil and allowed to stand for 7 days for extraction. The mixture was filtered using Whatmans filter paper No 1 and the filtrate was concentrated under are reduced pressure in an oven until a semi solid substance was obtained. This was dried inside the beaker under a controlled temperature (in an oven at 40^{o}C) to obtain solid extract (Jonathan and Fasidi, 2003).

Antimicrobial activities of mushroom extracts

The antimicrobial activities of ethanolic extracts were determined by filter paper disc methods of Norrel and Messely (1997). Sterile filter paper disc (1.5 cm diameter) was soaked with the test extracts and dried.

The disc was placed on fungus spp. seeded plate and placed in the refrigerator for 12 hours to allow the diffusion of the extract into the growing medium. The plates were incubated for at 25±1^{o} C in BOD incubator. Further, Fungus plates were incubated for 96 hrs for *Fusarium* fungus after which the Zone of inhibition was observed and measured. Each experiment was replicated 3 times along with control. The concentration of the extract used was 20 mg/ml and sterile distilled water was used in the dilution of extracts.

Results and Discussion

In antifungal activities against *Fusarium oxysporium,* Cadmium sulfate, Lead sulfate & Zinc sulfate with *Pleurotus pulmonarius* were found maximum effective in control of fungal species *Fusarium* spp., besides, Lead sulfate and cadmium sulfate was found moderately (11-13 mm) effective with *Lentinus squarrosulus* against *Fusarium.* While, Nickel sulfate, Ferrous sulfate, Molybdenum sulfate & Manganese sulfate (except Zinc sulfate in case with *Pleurotus pulmonarius*) were found weaker antifungal activity with *Pleurotus pulmonarius* & *Lentinus squarrosulus* against *Fusarium* spp.

Table.1 Antifungal activity of accumulated mushroom fruit body extracts on *Colletotrichum graminicola*

S. No.	Treatments (50 ppm)	Inhibition Zone (mm)	
		Lentinus squarrosulus	***Pleurotus pulmonarius***
1.	$PbSO_4$	10.5	18.1
2.	$ZnSO_4$	10.2	13.5
3.	$CdSO_4$	14.2	20.3
4.	$CuSO_4$	8.2	12.4
5.	$MnSO_4$	7.4	10.5
6.	$MoSO_4$	8.1	11.6
7.	$FeSO_4$	6.2	8.1
8.	$NiSO_4$	7.2	9.6
9.	Control (extract of fruiting body) without heavy metals	2.5	4.1
10.	Control	0	0
SEm±		2.38	0.36
CD at 5 %		7.02	1.07

Average of three replications

Table.2 Antifungal activity of accumulated mushroom fruit body extracts on *Alternaria solani*

S. No.	Treatments (50 ppm)	Inhibition Zone (mm)	
		Lentinus squarrosulus	***Pleurotus pulmonarius***
1.	$PbSO_4$	12.8	20.2
2.	$ZnSO_4$	8.3	10.5
3.	$CdSO_4$	13.1	21.5
4.	$CuSO_4$	9.5	13.3
5.	$MnSO_4$	7.1	9.7
6.	$MoSO_4$	7.4	10.1
7.	$FeSO_4$	8.6	9.4
8.	$NiSO_4$	8.3	10.7
9.	Control (extract of fruiting body) without heavy metals	4	5
10.	Control	0	0
SEm±		0.29	0.26
CD at 5 %		0.86	0.79

Average of three replications

Table.3 Antifungal activity of accumulated mushroom fruit body extracts on *Fusarium oxysporium*

S. No.	Treatments (50 ppm)	Inhibition Zone (mm)	
		Lentinus squarrosulus	***Pleurotus pulmonarius***
1.	$PbSO_4$	10.3	17.4
2.	$ZnSO_4$	7.8	12.3
3.	$CdSO_4$	12.2	18.1
4.	$CuSO_4$	8.1	11.6
5.	$MnSO_4$	5.6	8.3
6.	$MoSO_4$	8.2	9.1
7.	$FeSO_4$	6.4	8.6
8.	$NiSO_4$	5.3	8.7
9.	Control (extract of fruiting body) without heavy metals	3.3	4.1
10.	Control	0	0
SEm ±		0.15	0.34
CD at 5 %		0.44	1.00

Average of three replications
Inhibition Zone: 15 mm or greater-good antifungal activity; 11-14- moderate antifungal activity; 8-10- weak antifungal activity

Fig.1 Antifungal activity of accumulated mushroom fruit body extracts on *Colletotrichum graminicola*

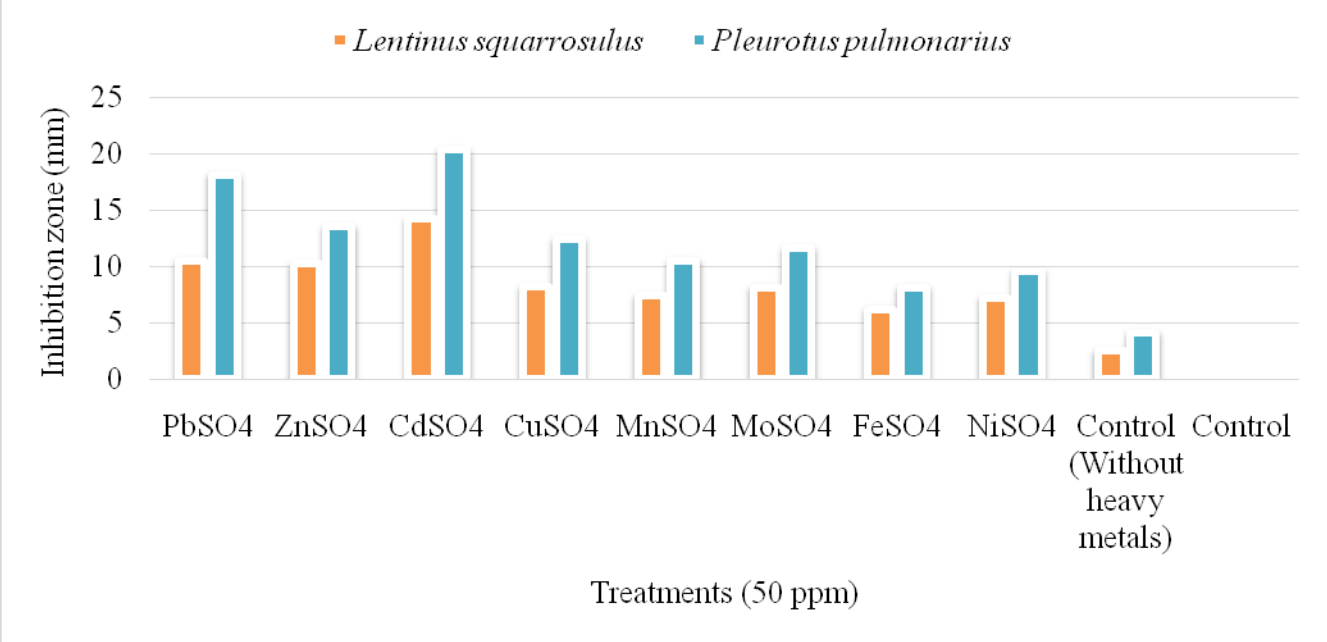


Fig.2 *Antifungal* activity of accumulated mushroom fruit body extracts on *Alternaria solani*

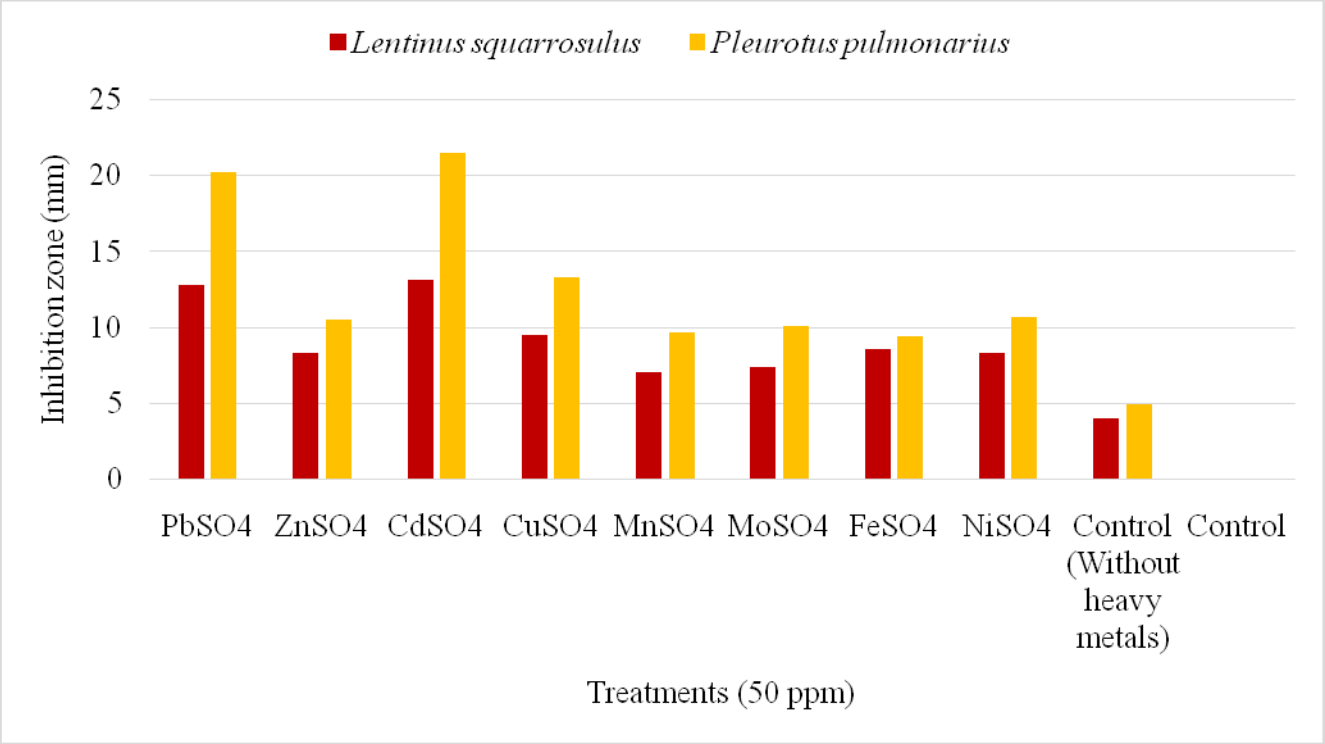
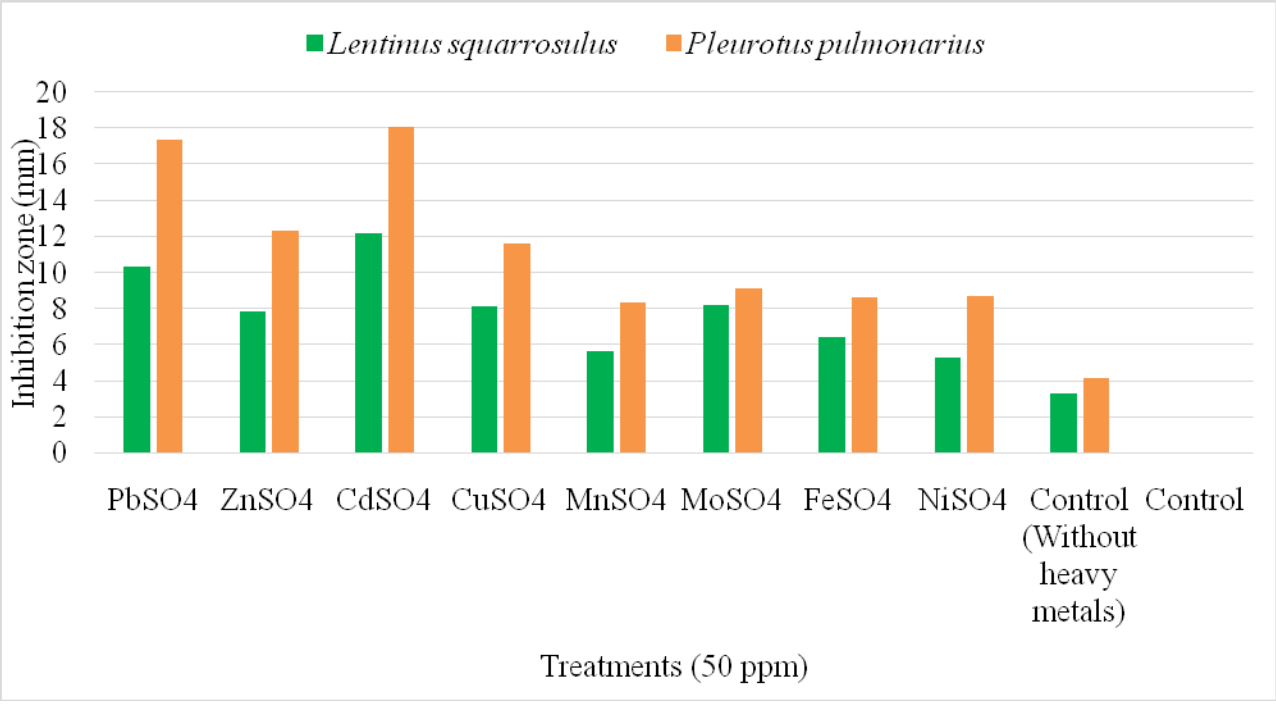


Fig.3 Antifungal activity of accumulated mushroom fruit body extracts on *Fusarium oxysporium*



In case *Alternaria* spp. antifungal activity, Lead sulfate & Cadmium sulfate were found maximum effective control of fungal species *Alternaria* with both studied mushroom species. Moreover, remaining heavy metals were found moderate (8-12 mm) in their antifungal activity against *Alternaria* spp. with only *Pleurotus pulmonarius* whereas these were found weaker (5-8.5 mm) effective with *Lentinus squarrosulus* against *Alternaria* spp.

In antifungal activities against *Colletotrichum* spp., Cadmium sulfate, Lead sulfate & Zinc sulfate were found maximum antifungal activities against *Colletotrichum* spp. with *Pleurotus pulmonarius* & *Lentinus squarrosulus*. While, Copper sulfate, Manganese sulfate, Molybdenum sulfate & Nickel sulfate were found moderate effective with *Pleurotus pulmonarius* against *Colletotrichum* spp. and weaker antifungal activity with *Lentinus squarrosulus* against *Colletotrichum* spp. The observation was supported by Chu *et al.,* (2005) which showed that antifungal peptide isolated from *Pleurotus ostreatus* found good inhibitory effect against *Fusarium oxysporum*. It is also evident in a study by Wang and Ng, (2004) and Ngai and Ng, (2004).

Antifungal protein is ribonuclease and Eryngin, an antifungal peptide isolated from *Pleurotus sajor* and *Pleurotus eryngi* which showed activity against *Fusarium oxysporum*. In addition, as there is always a need to develop new bio fungicides; some mushrooms extracts have been shown to act as biopesticides, supported by Imtiaj and Lee, (2007).

Antimicrobial activity of studied mushroom species increased with concentration of accumulated heavy metals. A good alternate option for plant pathogenic diseases, control with environment safety.

References

Anke, T. (1989). Basidiomycetes: A source for new bioactive secondary metabolites. *Prog. Ind. Microbiology*27: 51-57.

Breheret, S., T. Talou, S. Rapiorand J. M. Bessiere. (1997). Monoterpenes in the aromas of fresh wild mushrooms (Basidiomycetes). *Journal of Agriculture and Food Chemistry*45: 831-836.

Chu, K. T., X. Lixin and T. B. Ng (2005). Pleurostrin an antifungal peptide from the oyster mushroom. *Science Direct. Carbohydrate Polymers*58: 31–33.

Dahima, V., Anila Doshi and H. Singh. (2020). Screening of antifungal activity of *Pleurotus pulmonarius, P. florida* and *Shizophyllum commune*. *Int. J. Curr. Microbiol. App. Sci.* 9 (4): 997-1004.

Gupta, R., M. K. Khokhar and B. L. Roat (2018). Uptake and bioremediation of heavy metals through cultivated mushroom (*Calocybe indica*). *Ann. Pl. Protec. Sci.*26 (1): 175-180

Imtiaj, A. and T. S. Lee. (2007). Screening an antibacterial and antifungal activities from Korea wild mushrooms. *World J. Agric. Sci*.3: 316-321.

Janssens, L., H. L. Pooter, N. M. Schampand E. J. Vandamme. (1992). Production of flavors by microorganisms. *Process Biochemistry*27(2): 195-215.

Jonathan, S. G. and I. O. Fasidi (2003). Antibacterial activities of Nigerian edible macro fungi- *Lycoperdon pusilum*(Bat.Ex) and *Lycoperdon giganteus* (Pers.). *Af. J. Biomed.Res.*6: 85-90.

Lindequist, U., T. H. J. Niedermeyer and W. D. Julich. (2005). The Pharmacological potential of mushrooms. CAM2: 285-299.

Marumoto, R., D. Klostermeyer, W. Steglich,

A. Wunder and T. Anke. (1997). Phlebiachrysoic acids, new inhibitors of leukotriene biosynthesis from *Phlebia chrysocrea* (Basidiomycete). *LeibigsAnnalen-Recueil*2: 313-316.

Ngai, P. H. K. and T. B. Ng. (2004). A rib nuclease with antimicrobial, antimitogenic and anti-proliferative activities from the edible mushroom *Pleurotussajor caju*. *Peptides*25: 11-17.

Norrel, S. A. and K. E. Messley. (1997). Microbiology Laboratory Manual Principles and Applications. Prentice Hall, Upper Saddle River, New Jersey.

Quang, D. N., T. Hashimoto and Y. Asakawa. (2006). In edible mushrooms: A good source of biologically active substances. *Chem. Record.* 6: 79-99.

Wang, H. and T. B. Ng. (2004). Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii*. *Peptides*25: 1-5.

Wasser, S. P. and A. L. Weis. (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushroom: *A modern perpesctive. Crit.Rev. Immunol.*19:65-96.