ARTIGOS

Antifungal activity of cultivated oyster mushrooms on various agro-wastes

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ABSTRACT

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This study evaluated the antifungal activity of four fruiting bodies of oyster mushroom harvested from three agro-substrates *in vitro*. At three concentrations (2, 4 and 8 mg/disc), extracts discs of *Pleurotus ostreatus* (grey), *P. ostreatus* var. *florida*, *P. cornucopiae* var. *citrinopileatus* and *P. salmoneostramineus* were tested against three fungal pathogens: *Trichoderma harzianum* (after 2 days), *Verticillium* sp. and *Pythium* sp. (after 5 days) via the Disc Diffusion Method. The highest overall activity was by the extract disc Y2 (*P. cornucopiae* grown on M2 substrate; 70% wheat straw, 20% hardwood sawdust and 10% date palm fibers) and the lowest by Y1 (*P. cornucopiae* grown on wheat straw). The best inhibition zone was 16 mm toward *T. harzianum* by extract disc W2 (2 mg/disc) (*P. ostreatus* var. *florida* grown on M2 substrate), compared with 23 mm with Nystatin disc (100 U), followed 7 and 5 mm by P3 (*P. salmoneostramineus* grown on M3 substrate; 50% wheat straw, 30% hardwood sawdust and 20% date palm fibers) extract disc (8 mg/disc) against *Pythium* sp., and (4 mg/disc) against *Verticillium* sp., respectively.

Keywords: *Pleurotus* spp., agro-wastes, contaminated fungi, antifungal activity, bioactivity.

RESUMO

Owaid; M.N.; Al-Saeedi, S.S.S.; Al-Assaffii, I.A.A. A actividade anti-fúngica de cogumelos de ostra cultivada em várias agro-resíduos. ***Summa Phytopathologica,*** v.43, n.1, p.09-13, 2017.

Neste estudo, a actividade anti-fúngica de quatro corpos de frutificação do cogumelo ostra que colhidas a partir de três substratos agrícolas foi efetuada. Em três concentrações (2, 4 e 8 mg / disco), extraídos de discos de *Pleurotus ostreatus* (cinza), *P. ostreatus* var. *florida*, *P. cornucopiae* var. *citrinopileatus* e *P. salmoneostramineus,* testadas contra três fungos patogênicos: *Trichoderma harzianum* (após 2 dias), *Verticillium* sp. e *Pythium* sp. (após 5 dias), utilizando o método de difusão em disco. Em geral, a actividade mais elevada foi de Y2 (*P. cornucopiae* que cresceu sobre o substrato 70% de palha de trigo, 20% de serragem branca e 10% de fibras de palma) e a menor atividade foi de Y1 (*P. cornucopiae* que cresceu na palha de trigo). A melhor zona de inibição foi de 16 mm, em direção *T. harzianum* por W2 (*P. ostreatus* (branco), que cresceu sobre o substrato 70% palha de trigo, 20% serragem branca e 10% de fibras de tamara) (2 mg / disco), em comparação com 23 mm com disco de Nistatina (100 L), seguido 7 mm e 5 milimetros por P3 (*P. salmoneostramineus* que cresceram nas fibras de palma 50% solo de substrato de palha de trigo, 30% de serragem branca 20%) (8 mg / disco) contra *Pythium* sp. e (4 mg / disco) contra *Verticillium* sp., respectivamente.

Palavras-chave: *Pleurotus* spp., resíduos agrícolas, fungos contaminantes, atividade

The oyster mushroom, *Pleurotus* spp., is edible and belongs to the fungi kingdom, phylum Basidiomycota (32). About seventy species of *Pleurotus* spp. have been recorded and new species are being discovered. Many oyster mushrooms are primary decomposers of hardwood trees found worldwide (14). Thus, it can be cultivated on a wide variety of substrates containing lignin, cellulose and hemicellulose (26). It must obtain nutrients from such organic sources as dead organisms since they had absorbed nutrients after digesting large molecules into smaller units because of their secreted enzymes (10); thus, it has been grown in Iraq on various agro-wastes in the wild (18), or manually (5, 17) on cardboard (19, 20), date palm wastes (21), and tree sawdust (24).

Since ancient times, macrofungi have been used as a valuable food source and as traditional medicines around the world. The fungi constitute an important source for some compounds including enzymes and antibiotics (9). Consequently, the antimicrobial activity of various polysaccharides from medicinal mushrooms is being reevaluated in relation to their clinical efficacy, given that such compounds would be expected to function to ward off bacterial and fungal infections resistant to current antibiotics (31). Medicinal mushrooms are able to synthesize a great amount of secondary metabolites that present anti-tumoral, antiviral, anti-inflammatory (8), antibacterial, antifungal (23) and anti-yeast activities (25).

Currently, a large range of mushrooms species are grown in liquid media. The obtained mycelia used for various applications, such as obtaining dietary supplements, pharmaceutical applications, conversion of waste into biomass and production of enzymes (11). Akyuz et al. (3) reported that the secondary metabolism of *Pleurotus eryngii* was active against *Candida albicans*, *C. glabrata* and *Epidermophyton* spp. The methanolic extract of *Pleurotus* species demonstrated an inhibition in the growth of *C. albicans*, *C. glabrata*, species of *Trichophyton* and *Epidermophyton* (28). Mycelia and liquid filtrate of *Pleurotus* spp. showed variable inhibition activity against *Trichoderma harzianum*,

Pythium sp. and *Verticillium* sp. (5).

The *T. harzianum* isolated from a farm for *Agaricus bisporus* cultivation has caused losses in mushroom production, while it and other species caused contamination of spawn and agro-substrates (6). At the same time *T. harzianum* has been reported as an effective biocontrol agent against several plant fungal diseases (29, 30, 34). Furthermore, *T. harzianum* may not be able to cause economic loss in the commercial cultivation of *P. tuberregium* since mycelium of *P. tuberregium* was able to overgrow completely in the presence of pathogenic fungi (7).

An antifungal peptide was isolated from fruiting bodies of the mushroom *P. eryngii* which inhibited mycelial growth of pathogenic fungi (35). Gregori et al. (11) reported that production of *Pleurotus* spp. mycelial biomass and valuable polysaccharides in submerged liquid fermentation depends on the species used, growth parameters, growth timing and their nutritional requirements. Antifungal agents such as chitinase and protease from culture filtrate of *P. ostreatus*, *P. florida* and *P. sajor-caju* were able to exert successful control of soil fungi *in vitro* (12).

In another study, production of *p*-anisaldehyde by *P. ostreatus* was observed as a defense mechanism against other organisms by providing antibacterial and antifungal activity (27). The presence of tannins, saponins and flavonoids in *P. ostreatus* var. *florida* may be responsible for the positive antifungal activity against *Trichoderma* sp. in aqueous extracts (16). Antifungal activity of *Pleurotus* spp. has been observed in isolated compounds such as unsaturated fatty acids in mycelia and cultured liquid extracts (9), and was attributable to some factors that affected mycelial growth such as the temperature, pH and the culture medium (1).

However, this article showed anti-fungal activity of extracts from four species of cultivated oyster mushrooms on three agro-wastes against three pathogenic fungi, namely *Trichoderma harzianum*, *Verticillium* sp. and *Pythium* sp. The importance of the current study is due to its contribution to knowledge on the action of mycelia of oyster mushroom species harvested from different agro-substrates against some fungal pathogens.

MATERIALS AND METHODS

Fungal Strains

The three fungal pathogens strains used in this study – namely *Trichoderma harzianum*, *Verticillium* sp. and *Pythium* sp. – were obtained from the Plant Pathology and Fungi Laboratory, College of Science, University of Anbar, Iraq. They were sub-cultured on potato dextrose agar (PDA) medium and stored at 25 °C for this study.

Samples of Mushrooms

Twelve extracts of fruiting bodies of oyster mushroom species were investigated. Fruiting bodies of *Pleurotus ostreatus* (grey oyster), *P. ostreatus* var. *florida*, *P. cornucopiae* var. *citrinopileatus* (bright yellow oyster) and *P. salmoneostramineus* (pink oyster) were harvested from three agro-substrates (Table 1) from the Department of Biology in the College of Science, University of Anbar, Iraq.

Table 1. Ingredients of agro-substrates

Mixtures	Ingredients of substrates		
	Wheat straw	Hardwood sawdust	Date palm fibers
Mixture 1 (M1)	100%	-	-
Mixture 2 (M2)	70%	20%	10%
Mixture 3 (M3)	50%	30%	20%

Bioactivity of oyster mushroom extracts

Extraction and preparation of extract disc

The extracts were prepared from dried fruiting bodies at 45 °C in an oven then powdered. The powder was then placed in distillled water at the same ratio (1:10) for each sample, and shaken in a shaker at 150 cycle/min at 25 °C for 24 h (4). Aqueous extracts were filtered using Whatman No.1 filter paper and dried in glass dishes in hot air in an oven at 40 °C until had obtainment of a thickened mass, weighed then sterilized using Mollipore Sryinge filter 0.20 µ.

Blank discs (6 mm) were prepared from sterilized Whatman No. 1. Then determined amount of aqueous extract from each one separately was placed on blank discs for producing extract disc concentrations 2, 4 and 8 mg/disc, transfered to incubator until dried, marked and stored in a sterilized vial at 4 °C until use. By the same method, antibiotic Nystatin disc 100 U was prepared and used as control.

Disc Diffusion Method

The antifungal effects of the oyster mushrooms extracts were determined using disc diffusion method (33). Three discs of extracts were placed in the petri dish of Mueller-Hinton Agar, and then left 30 min to spread the extracts in medium. There were inoculated by disc of old seven day pathogenic fungi centrally, incubated at 28 °C to enable monitoring of their development; then the zone of inhibition was measured.

Statistical Analysis

Experimental values are given as means. Statistical significance was determined by Two Way ANOVA (no blocking) with three replications. Data were analyzed and graph was constructed by statistical program, GenStat Discovery Edition computer program version 7 DE3 and Microsoft Excel version 2010. Differences at $P < 0.05$ were considered to be significant.

RESULTS

The action of oyster mushroom extract was variable (Table 2). After 2 days, the best inhibition zone against *Trichoderma harzianum* was 16 mm by the W2 extract disc (2 mg/disc) (*Pleurotus ostreatus* var. *florida* grown on M2 substrate; 70% wheat straw, 20% hardwood sawdust and 10% date palm fibers), followed 14 mm by W1 extract disc (4 and 8 mg/disc) (*P. ostreatus* var. *florida* grown on M1; wheat straw) compared with 23 mm with antibiotic Nystatin disc (100 U). The lowest inhibition zone was 1 mm by the extract disc P2 (4 and 8 mg/disc) (*P. salmoneostramineus* grown on M2). No inhibition zone found in extract discs of W3 (2 mg/disc) (*P. ostreatus* var. *florida* grown on M3 substrate; 50% wheat straw, 30% hardwood sawdust and 20% date palm fibers), Y1 (2 mg/disc) (*P. cornucopiae* grown on M1) and P3 (2, 4 and 8 mg/disc) (*P. salmoneostramineus* grown on M3).

As to *Verticillium* sp., after 5 days, the best inhibition zone was 5 mm by the extract disc P3 (4 mg/disc), followed 4 mm by P3 (2 and 8 mg/disc) and Y3 (*P. cornucopiae* grown on M3) (8 mg/disc), while some concentrations of G3 did not present any anti-fungal effect (*P. ostreatus* (grey) grown on M3), W1, W2, Y1, Y3, P1 and P2 extract

Table 2. Inhibition zones of fungal pathogens by oyster mushroom extract discs

Mushroom species	Extract discs	mg/disc	*T. harzianum* [a]	*Verticillium* sp. [b]	*Pythium* sp. [b]
Pleurotus ostreatus (grey)	**G1**	**2**	5	2	3
		4	6	1	3
		8	10	3	3
	G2	**2**	3	1	0
		4	4	3	0
		8	4	3	0
	G3	**2**	6	0	0
		4	6	1	1
		8	5	1	1
Pleurotus ostreatus var. *florida*	**W1**	**2**	4	2	0
		4	14	0	0
		8	14	0	0
	W2	**2**	16	0	0
		4	4	0	0
		8	2	1	0
	W3	**2**	0	2	1
		4	2	1	1
		8	2	2	1
Pleurotus cornucopiae var. *citrinopileatus*	**Y1**	**2**	0	0	0
		4	2	0	1
		8	4	0	1
	Y2	**2**	14	2	4
		4	4	2	4
		8	2	2	4
	Y3	**2**	2	2	0
		4	2	0	0
		8	2	4	5
Pleurotus salmoneostramineus	**P1**	**2**	2	0	3
		4	4	2	3
		8	2	1	6
	P2	**2**	2	0	1
		4	1	3	3
		8	1	2	2
	P3	**2**	0	4	7
		4	0	5	5
		8	0	4	2
control	**Nystatin**	**100 U**	23	5.8	12

Notes: **([a])** after 2 days, **([b])** after 5 days. **G1**: extract disc of *Pleurotus ostreatus* (grey) harvested from M1 substrate (wheat straw), **G2**: extract disc of *P. ostreatus* (grey) harvested from M2 substrate (70% wheat straw, 20% hardwood sawdust and 10% date palm fibers), **G3**: extract disc of *P. ostreatus* (grey) harvested from M3 substrate (50% wheat straw, 30% hardwood sawdust and 20% date palm fibers). **W1**, **W2** and **W3**: extract disc of *P. ostreatus* var. *florida* harvested from M1, M2 and M3 substrates, respectively. **Y1**, **Y2** and **Y3**: extract disc of *P. cornucopiae* harvested from M1, M2 and M3 substrates, respectively. **P1**, **P2** and **P3**: *P. salmoneostramineus* extract disc harvested from M1, M2 and M3 substrates, respectively.

discs. Whereas Nystatin disc (100 U) showed 5.8 mm as its greatest effect, *Pythium* sp. was sensitive at all concentrations of the extracts discs G2, W1 and W2, and at some concentrations of extract discs G3, Y1 and Y3. The best performance was by the extract disc P3 (8 mg/ disc) which produced a 7 mm inhibition zone, followed by 6 mm by P1 (8 mg/disc) and 5 mm by P3 (4 mg/disc) and Y3 (8 mg/ disc). All of these findings are displayed in Table 2.

Overall, the highest extract-disc activity was 4.22 mm by Y2, followed by 4, 3.78 and 3 mm for the respective extract discs G1, W1 and P3, while the lowest activity was 0.89 mm by Y1 significantly (P<0.05), followed by 1.33 mm and 1.67 mm, respectively, by W3 and P2, as shown in Figure 1.

According to Figure 2, the fungus *T. harzianum* presented greater sensitivity toward oyster mushroom extracts than other fungal

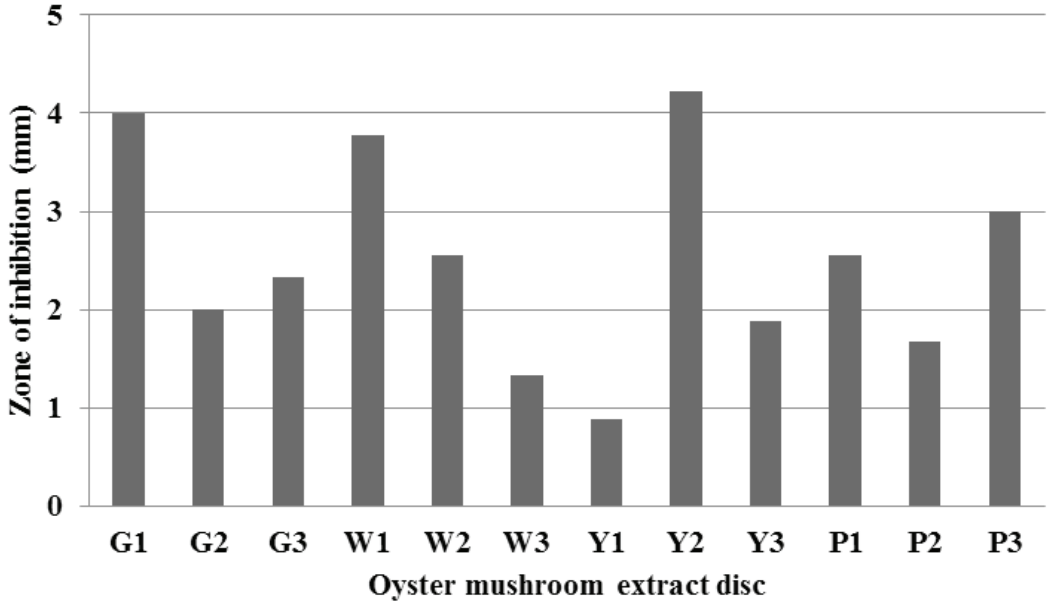

Figure 1. Overall comparison among inhibitors of oyster mushroom extract discs:
LSD ($P< 0.05$) = 2.092. **G1**: extract disc of *Pleurotus ostreatus* (grey) harvested from M1 substrate (wheat straw), **G2**: extract disc of *P. ostreatus* (grey) harvested from M2 substrate (70% wheat straw, 20% hardwood sawdust and 10% date palm fibers), **G3**: extract disc of *P. ostreatus* (grey) harvested from M3 substrate (50% wheat straw, 30% hardwood sawdust and 20% date palm fibers). **W1**, **W2** and **W3**: extract disc of *P. ostreatus* var. *florida* harvested from M1, M2 and M3 substrates, respectively. **Y1**, **Y2** and **Y3**: extract disc of *P. cornucopiae* harvested from M1, M2 and M3 substrates, respectively. **P1**, **P2** and **P3**: extract disc of *P. salmoneostramineus* harvested from M1, M2 and M3 substrates, respectively.

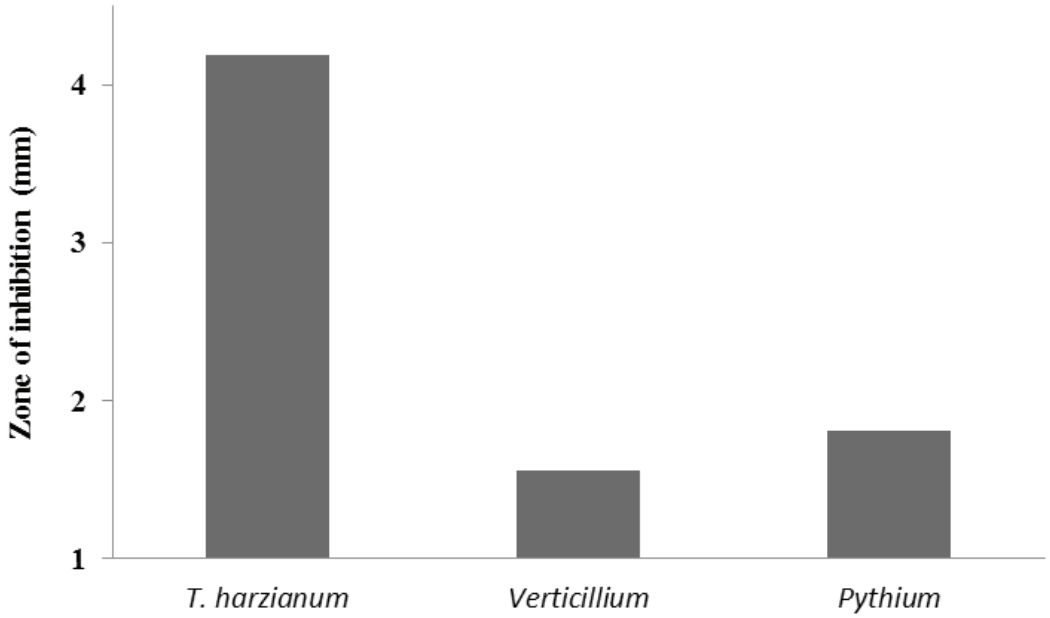

Figure 2. Sensitivity Average of fungal pathogens toward oyster mushroom extracts
LSD ($P< 0.05$) = 1.046. Averages of *T. harzianum* after 2 days, versus others after 5 days.

pathogens, significantly ($P<0.05$), on average 4.19 mm, compared with 1.81 mm and 1.56 mm produced by *Pythium* sp. and *Verticillium* sp., respectively.

DISCUSSION

The influence of twelve extracts from four *Pleurotus* spp. fruiting bodies showed efficaciousness against three plant pathogenic fungi because of differences in agro-waste sources used as substrates for production of fruiting bodies (5), as shown in Table 2, which may arise from the genetic structure of mushroom species and their physical, bioactive and biochemical constituents, and chemical differences of mushroom extracts, solvents and test microorganisms that another research study has shown clearly in comparison to other mushroom species (2).

Furthermore, some mushroom extracts contain nutrients besides antagonistic matter and, therefore, produce no effect in some cases (36). Bioactivity of their extracts may be attributable to their compounds, with wide ranging antimicrobial activity, which could be isolated from many mushrooms species (27). The differences of anti-fungal activities of varieties of these oyster mushroom extracts may be due to the genetic characteristics of oyster mushroom species that lead to alterations in chemical composition (13) that vary according to type of fungal product (27) such as isolated simple indolone from *P. salmoneostramineus* as a glycoprotein conjugate by aqueous extraction (15), anti-fungal agents like chitinase and protease from *P. ostreatus* and *P. florida* extracts against soil fungi (12) and *p*-anisaldehyde from *P. ostreatus* (27). In most aspects the fungus *T. harzianum* is more sensitive than other fungal pathogens due to the speed of its mycelial growth, which produced a clear inhibition zone in two days instead of the five days required by the others (5).

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