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Antifungal Activities of Some *Pleurotus* Species (Higher Basidiomycetes)

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Abstract

This study investigated the antifungal activities of 4 *Pleurotus* spp. (oyster mushrooms) against 3 pathogenic fungi: *Trichoderma harzianum*, *Verticillium* sp., and *Pythium* sp. The antifungal activity of the mycelia of oyster mushrooms was tested against 3 pathogenic fungi grown in fresh PDA medium using dual culture testing. The antifungal activities of the culture filtrates of oyster mushrooms were tested in solid and liquid media. The highest inhibition activity, of 55.56 %, was observed with *P. salmoneostramineus* against *Verticillium* sp., while the least percent inhibition of 46.15 % was with the mycelia of *Pleurotus ostreatus* against *T. harzianum*. Then, *Pleurotus ostreatus* grew over the mycelia of *Pythium* sp. in 5.33 days, while *P. cornucopiae* var. *citrinopileatus* mycelia did not grow over the mycelia of *Verticillium* sp. and *T. harzianum*. Antifungal activity of the culture filtrate of *Pleurotus* spp. on agar media was variable. The highest inhibition was 12.33 %, followed 11.60 % for *P. salmoneostramineus* and *P. ostreatus* against *Verticillium* sp. and *T. harzianum*, respectively, whereas lower inhibitions at 1.34 and 1.75 % were observed for the culture filtrates of *P. cornucopiae* and *P. ostreatus* against *Verticillium* sp. and *Pythium* sp., respectively. In liquid media, the highest inhibition was 55 and 50 % with *P. ostreatus* and *P. salmoneostramineus* culture filtrates against *T. harzianum* and *Verticillium* sp., respectively, whereas the lowest inhibition was 3.33 % in the case of *P. ostreatus* against *T. harzianum*.

Keywords: Culture filtrate, inhibition, mycelia, pathogenic fungi, *Pleurotus* spp.

Introduction

Macrofungi have long been used as a valuable food source and as traditional medicines around the world since ancient times [1,2]. Both fruiting bodies and the mycelia of oyster mushroom contain compounds with wide ranging antimicrobial activity, which could be isolated from many medicinal mushrooms species and be of benefit to plants and humans [3,4]. As an edible mushroom, the oyster mushroom *Pleurotus* spp. can be cultivated on a wide variety of agricultural substrates containing lignin, cellulose, and hemicellulose [5-9]. *Trichoderma harzianum* is the most dominant contaminant and causes extensive losses in the cultivation of the oyster mushroom and white button mushroom species. Besides *T. harzianum*, other *Trichoderma* species are common contaminants of mushroom spawn, compost, and wood in commercial mushroom growing facilities [10,11]. However, *T. harzianum* has been reported to

be an effective bio-control agent for several plant fungal diseases [12]. Further, *T. harzianum* may not be able to cause economic loss in the commercial cultivation of *P. tuberregium*, since mycelium of *P. tuberregium* is able to overgrow completely even in the presence of pathogenic fungi [13]. High temperature typically stimulates the growth rate and antagonistic activity of *P. tuberregium* against *Fusarium culmorum* and *T. harzianum*. The growth of *Pleurotus ostreatus* and *Pleurotus florida* is less affected by cypress leaf extract compared to the *Trichoderma* species, while the culture filtrate of alcoholic and aqueous extracts from *P. ostreatus*, *P. florida*, and *Pleurotus sajor-caju* were active against other soil fungi [14,15].

Some strains of *T. harzianum* and other plant pathogenic fungi are inhibited by the mycelia of some *Pleurotus* species [11,16] and *Flammulina velutipes* [17] in dual cultures. An antifungal peptide was isolated from fruiting bodies of the mushroom *Pleurotus eryngii*. The peptide, known as eryngin, inhibited the mycelial growth of pathogenic fungi [18]. Antifungal agents, like chitinase and protease from culture filtrates of *P. ostreatus*, *P. florida*, and *P. sajor-caju*, could successfully control soil fungi *in vitro* [15].

Although fruiting bodes of oyster mushrooms have good nutritional value [19]. Gregori *et al.* [20] reported that the 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. Polysaccharide production in a submerged culture of *Pleurotus ostreatus* exhibited antioxidant activity *in vitro* [21]. In another study, the production of *p*-anisaldehyde by *P. ostreatus* was observed as a defence mechanism against other organisms, providing antibacterial and antifungal activity [22]. The presence of tannins, saponins, and flavonoids in *P. florida* may be responsible for the positive antifungal activity against *Trichoderma* sp. in methanol and water extracts [23]. Antifungal activity of *Pleurotus* spp. has been observed in isolated compounds, such as unsaturated fatty acids in mycelium and culture liquid extracts [24]. Antifungal activity of *Pleurotus* spp. culture filtrates may be due to some factors which affect the growth of mycelium, such as temperature, pH, and medium [25]. Also, *Pleurotus cornucopiae* had a potential effect against *Candida* sp. through its silver nanoparticles [26].

The present study, therefore, seeks to access the antifungal activities of the mycelia of 4 *Pleurotus* spp. against 3 pathogenic fungi, viz., *Trichoderma harzianum*, *Verticillium* sp., and *Pythium* sp. The importance of the current study is due to the increasing use of spent mushroom substrate for the biological control of plant and button mushroom (*Agaricus bisporus*) pathogens.

Materials and methods

Fungal Strains

Four oyster mushroom species were investigated in the current study. *Pleurotus ostreatus* (grey oyster), *Pleurotus ostreatus* (white oyster), *Pleurotus cornucopiae* var. *citrinopileatus* (bright yellow oyster), and *Pleurotus salmoneostramineus* (pink oyster) were obtained from the Mushroom Box Company, Monmouth, UK, in the form of spawn. They were sub-cultured on potato dextrose agar (PDA) medium and stored at 25±2 °C. Three pathogenic fungal strains used in this study (*Trichoderma harzianum*, *Verticillium* sp., and *Pythium* sp.) were obtained from the Plant Pathology and Fungi Laboratory, College of Science, University of Anbar, Iraq.

Interaction between the mycelia of *Pleurotus* spp. and pathogenic fungi

The mycelia of oyster mushrooms were tested for antifungal activity against the 3 pathogenic fungi in fresh PDA medium using dual culture testing. The prepared PDA medium was autoclaved at 121 °C for 15 min and then poured into 9 mm Petri dishes. Culture disks measuring 5 mm were made in the Petri dishes for each pathogenic fungus. A culture plug of 7-day-old pathogenic fungi was placed 3 cm away from the mushroom culture plug. The plates were then incubated for 7 days at 25±1 °C. Plates were checked and the inhibition zone measured and recorded by comparing it with the control plates [27]. The number of days taken to overgrow the pathogens was recorded.

Antifungal activities of culture filtrate of oyster mushrooms

1. Collection of liquid culture filtrate

Mushrooms were cultured in 50 ml of potato dextrose broth (PDB) using 10-day-old 5 mm disks followed by incubation at 25±1 °C and observed daily for 20 days [28]. The liquid cultures were filtered through Whatman No. 1 filter paper twice and pH adjusted to 7 by HCl (1N). The mushroom culture filtrate used is referred to as antifungal agent in this study, unless otherwise stated.

2. Percent inhibition of mycelial growth and weight (PIMG) and (PIMW)

Mushroom culture filtrates were diluted with PDB medium separately to 50 % (v/v). Agar 1.5 % was added and then autoclaved at 121 °C for 25 min. Fresh PDA plates were used as control. The 7-day-old cultures of 3 pathogenic fungi were placed in the center of the plates and incubated at 25 °C for 10 days to detect inhibitory activity by measuring the radial growth on culture filtrate (R2) and the radial growth on a fresh PDA plate as control (R1). The experiment was carried out in replicates. The PIMG was calculated by using the following equation [29]:

$$PIMG = \{(R1-R2)/R1\} \times 100. \quad (1)$$

Determination of the percent inhibition of the mycelial weight (PIMW) of the 3 pathogenic fungi was done by diluting culture filtrates without agar following autoclaving. Inoculation of fungi was done separately using 50 ml of liquid medium in 250 ml of conical flask at 25 °C for 10 days, while fresh PDB was used as control treatment. Consequently, the mycelium was harvested by filtering through previously dried and weighed mycelium. Mycelium was then dried at 65 °C, and the dry weight was calculated using the same formula [29].

Statistical analysis

Statistical significance was determined by using a 2 way analysis of variance (ANOVA) by implementing the GenStat Discovery Edition computer program version 7 DE3 (VSN International Ltd., UK). Significant differences at $P < 0.05$ were considered. All the experiments were done in 3 replicates.

Results

Interaction between *Pleurotus* spp. and pathogenic fungi mycelia using dual culture testing

The percent inhibition of *Pleurotus* spp. mycelia recorded against the 3 pathogenic fungi viz., *Trichoderma harzianum*, *Verticillium* sp., and *Pythium* sp. is presented in **Table 1**. It is evident from the table that a significant difference ($P < 0.05$) was observed between the percent inhibitions of different pathogenic fungi. The overall inhibition percentage was between 46 and 56 %. The maximum percent of inhibition (55.56 %) was shown by the mycelia of *P. salmoneostramineus* against the fungus *Verticillium* sp. An inhibition percentage of 46.15 % by the mycelia of *P. ostreatus* against *T. harzianum* was the lowest percentage recorded in the study. From comparison of all the 4 *Pleurotus* spp., *P. salmoneostramineus* showed better inhibition against all the 3 pathogenic fungi.

Table 1 Percent inhibition of *Pleurotus* spp. mycelia against pathogenic fungi on PDA after 4 days by dual culture testing in Petri dishes.

Pathogenic fungi	*Pleurotus* spp. mycelia			
	P. ostreatus **(grey)**	***P. ostreatus*** **(white)**	***P. cornucopiae*** **(yellow)**	***P. salmoneostramineus*** **(pink)**
T. harzianum	46.15	47.18	49.74	50.77
Verticillium sp.	49.21	54.50	52.38	55.56
Pythium sp.	50.79	54.50	52.91	54.50
LSD (P < 0.05)	**2.246**			

Legend: LSD: Least Significant Difference (Probability less than 0.05)

Growth of *Pleurotus* spp. mycelia over pathogenic fungi mycelia

The growth pattern of *Pleurotus* spp. mycelia over pathogenic fungi mycelia using dual culture testing is shown in **Figure 1**. *Pleurotus ostreatus* (G) and *P. salmoneostramineus* (PK) showed significant differences ($P < 0.05$) in their growths over *Pythium* sp. They overgrew in a significantly lesser period of 5.33 days, while *P. ostreatus* (G) overgrew over *Verticillium* sp. in about 7.33 days. *T. harzianum* was inhibited by all *Pleurotus* species in dual culture, but the *Pleurotus* species could not overgrow in the presence of this pathogen (**Figures 1 - 2** and **Table 1**).

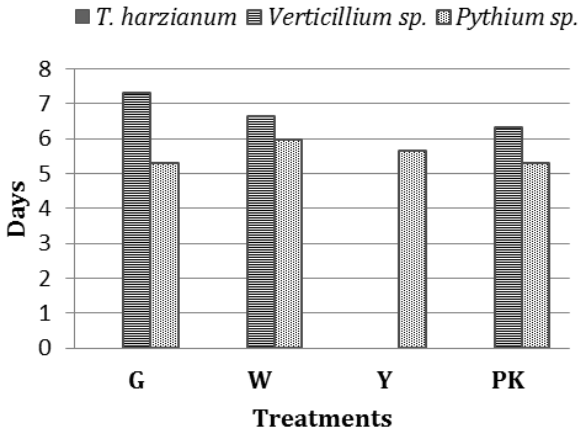

Figure 1 Times of days for overgrowth of *Pleurotus* spp. mycelia in presence of pathogenic fungi mycelia in dual culture.
Legend: G: *P. ostreatus*, W: *P. ostreatus*, Y: *P. cornucopiae* var. *citrinopileatus* and PK: *P. salmoneostramineus*

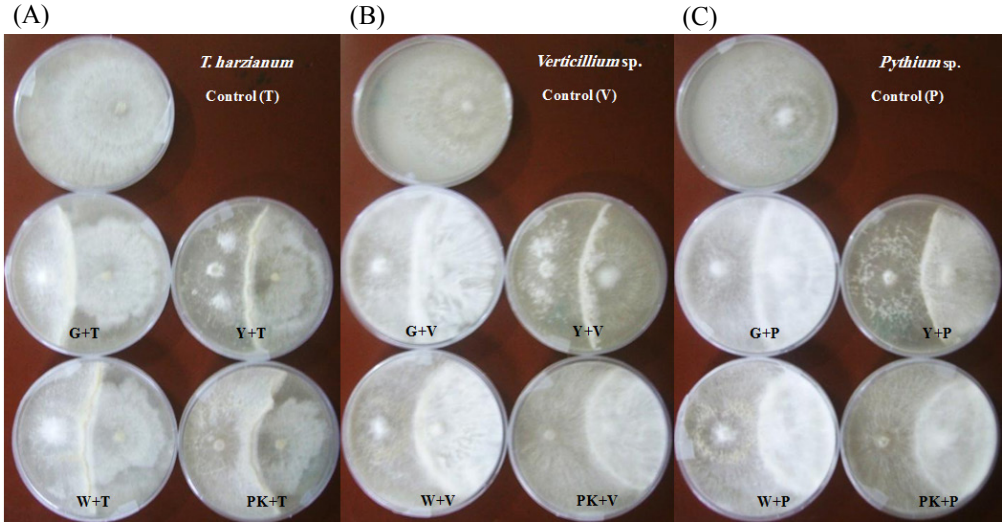

Figure 2 Overgrowth of *Pleurotus* spp. mycelia in presence of pathogenic fungi mycelia in dual culture after 7 days.
Legend: (A) G+T: *P. ostreatus* with *T. harzianum,* W+T: *P. ostreatus* with *T. harzianum,* Y+T: *P. cornucopiae* with *T. harzianum,* PK+T: *P. salmoneostramineus* with *T. harzianum.* (B) G+V: *P. ostreatus* with *Verticillium,* W+V: *P. ostreatus* with *Verticillium,* Y+V: *P. cornucopiae* with *Verticillium,* PK+V: *P. salmoneostramineus* with *Verticillium.* (C) G+P: *P. ostreatus* with *Pythium,* W+P: *P. ostreatus* with *Pythium,* Y+P: *P. cornucopiae* with *Pythium,* PK+P: *P. salmoneostramineus* with *Pythium.*

Influence of *Pleurotus* spp. culture filtrate on mycelial growth rate of fungi in solid medium

Significantly ($P < 0.05$) lower growth was recorded (13.03 mm/day with *P. salmoneostramineus* culture filtrate, followed by 13.93 mm/day by *P. ostreatus*) against *Verticillium* sp. The highest growth rate was recorded as 15.93 mm/day with *T. harzianum* by the culture filtrate of *P. salmoneostramineus,* followed by 15.67 and 15.60 mm/day by *P. cornucopiae* and *P. ostreatus*, respectively, in the presence of the same pathogen. Likewise, a 15.07 mm/day growth rate for *Pythium* sp. with *P. ostreatus* culture filtrate was observed. Generally, all culture filtrates indicated low growth averages compared to the control (**Table 2**). **Figure 3** exhibited normal growth, except for *T. harzianum,* which had low growth with all *Pleurotus* species.

Table 2 Mycelial growth rate of fungi on solid medium of *Pleurotus* spp. culture filtrate after 5 days (mm/day).

Pathogenic fungi	PDA	Solid medium of *Pleurotus* culture filtrate			
		***P. ostreatus* (grey)**	***P. ostreatus* (white)**	***P. cornucopiae* (yellow)**	***P. salmoneostramineus* (pink)**
T. harzianum	16.67	14.73	15.60	15.67	15.93
Verticillium sp.	14.87	14.73	13.93	14.67	13.03
Pythium sp.	15.33	15.07	14.47	14.63	14.30
LSD ($P < 0.05$)	**0.6771**				

Legend: LSD: Least Significant Difference (Probability less than 0.05)

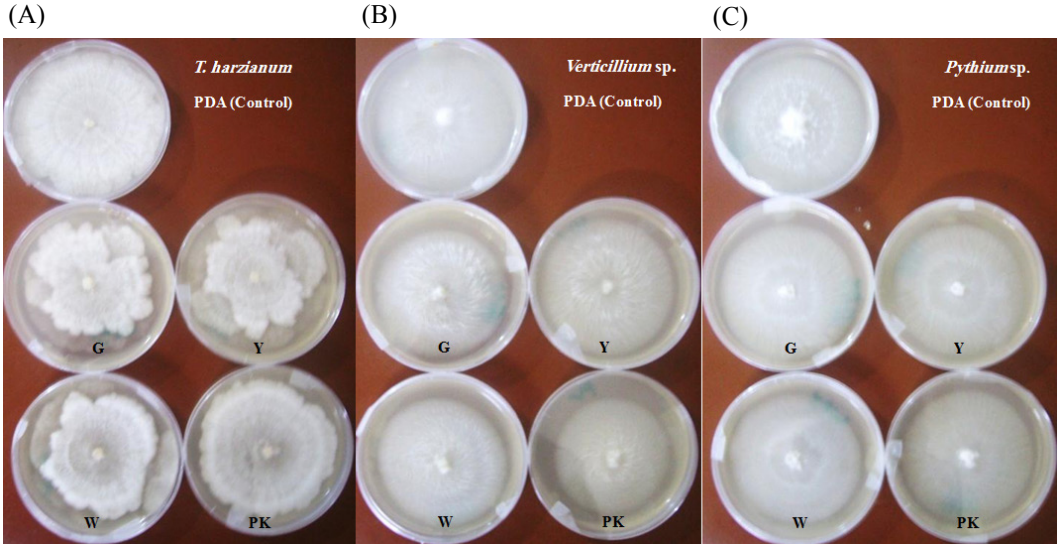

Figure 3 Mycelial growth pattern of pathogenic fungi on solid medium of culture filtrate of *Pleurotus* sp. after 5 days.

Legend: (A) *Trichoderma harzianum* (B) *Verticillium* sp. (C) *Pythium* sp., PDA: Fresh potato dextrose agar 100 % (control), G: Solid medium of culture filtrate of *P. ostreatus* (grey) with fresh potato dextrose broth 50%:50% (v/v), W: Solid medium of culture filtrate of *P. ostreatus* (white) with fresh potato dextrose broth 50%:50% (v/v), Y: Solid medium of culture filtrate of *P. cornucopiae* with fresh potato dextrose broth 50%:50% (v/v), PK: Solid medium of culture filtrate of *P. salmoneostramineus* with fresh potato dextrose broth 50%:50% (v/v).

Percent inhibition of *Pleurotus* spp. culture filtrate by mycelial growth rate of pathogenic fungi in solid medium

The inhibition percentage of different *Pleurotus* species culture filtrate on pathogenic fungi in solid medium was observed (**Table 3**). The highest inhibition rate of 12.33 %, followed by 11.60 %, was observed for *P. salmoneostramineus* and *P. ostreatus* against *Verticillium* sp. and *T. harzianum*, respectively, whereas lower inhibitions of 0.90 and 1.34 % were observed for *Verticillium* sp.against a solid medium of *P. ostreatus* and *P. cornucopiae* culture filtrate, respectively.

Table 3 Percent inhibition of *Pleurotus* culture filtrate on pathogenic fungi in agar medium after 5 days.

Pathogenic fungi	Solid medium of *Pleurotus* culture filtrate			
	P. ostreatus **(grey)**	***P. ostreatus*** **(white)**	***P. cornucopiae*** **(yellow)**	***P. salmoneostramineus*** **(pink)**
T. harzianum	11.60	6.40	6.00	4.40
Verticillium sp.	0.90	6.28	1.34	12.33
Pythium sp.	1.75	5.66	4.57	6.75
LSD (P < 0.05)	**1.634**			

Legend: LSD: Least Significant Difference (Probability less than 0.05)

Evaluation of *Pleurotus* spp. culture filtrate on biomass of pathogenic fungi

The influence of the culture filtrate of *Pleurotus* species on the dry weight of pathogenic fungi mycelia in culture filtrate broth compared to the control (PDB) is presented in **Table 4**. The highest dry weight of 220 mg was observed by *Verticillium* sp. in fresh PDB, followed by 210 and 200 mg for *Pythium* sp. and *T. harzianum*. *P. ostreatus* and *P. cornucopiae* exhibited dry weights of 193.33 and 190 mg for *T. harzianum* and *Verticillium* sp., respectively. The lowest dry weights of 90 and 110 mg were observed in *P. ostreatus* broth for *T. harzianum* and *Verticillium* sp., respectively.

Percent inhibition of *Pleurotus* spp. culture filtrates on dry weight pathogenic fungi in liquid medium

The percent inhibition of *Plerotus* spp. culture filtrate against pathogenic fungi in liquid medium after 10 days is presented in **Table 5**. The highest inhibition of 55 % was recorded in the filtrate of *P. ostreatus* against *T. harzianum* in liquid medium, followed by 50 and 43.94 % in *P. salmoneostramineus* and *P. ostreatus* filtrate observed against *Verticillium* sp. However, lesser inhibitions (3.33 % and 12.70 %) were recorded in *P. ostreatus* filtrate against *T. harzianum* and *Pythium* sp., respectively, followed by 13.64 and 15.00 % in the culture filtrate of *P. cornucopiae* against *Verticillium* sp. and *T. harzianum*, respectively.

Table 4 Effect of *Pleurotus* spp. culture filtrate on dry weight of pathogenic fungi in broth after 10 days (mg/50 ml).

Pathogenic fungi	PDA	Liquid medium of *Pleurotus* spp. culture filtrate			
		P. ostreatus **(grey)**	***P. ostreatus*** **(white)**	***P. cornucopiae*** **(yellow)**	***P. salmoneostramineus*** **(pink)**
T. harzianum	200.00	90.00	193.33	170.00	133.33
Verticillium sp.	220.00	123.33	150.00	190.00	110.00
Pythium sp.	210.00	140.00	183.33	143.33	170.00
LSD (P < 0.05)	**1.0249**				

Legend: LSD: Least Significant Difference (Probability less than 0.05)

Table 5 Percent inhibition of *Pleurotus* spp. culture filtrate against pathogenic fungi in liquid medium after 10 days.

Pathogenic fungi	Liquid medium of *Pleurotus* spp. culture filtrate			
	P. ostreatus **(grey)**	***P. ostreatus*** **(white)**	***P. cornucopiae*** **(yellow)**	***P. salmoneostramineus*** **(pink)**
T. harzianum	55.00	3.33	15.00	33.33
Verticillium sp.	43.94	31.82	13.64	50.00
Pythium sp.	33.33	12.70	31.74	19.05
LSD (P < 0.05)	**2.439**			

Legend: LSD: Least Significant Difference (Probability less than 0.05)

Discussion

The secondary metabolism of oyster mushroom mycelia is important in preventing pathogenic fungal growth. **Figure 1** shows the effect of *Pleurotus* spp. filtrate against the growth of fungi. The filtrate of *P. salmoneostramineus* was the most effective compared with other filtrates. *Trichoderma harzianum* had low sensitivity, due to the genetic characters of species of oyster mushroom which lead to different secondary metabolisms. Metabolism affected on bioactivity of these filtrates. The chemical composition of the broth of oyster mushroom varies according to the type of fungus product [22].

Although sterilization of culture filtrates of oyster mushroom using the autoclaving process was performed, these filtrates showed inhibitory factors toward *Trichoderma* sp. High pressure thermal exposure in fungal filtrates may bring about irreversible or reversible, complete or partial, enzyme inactivation, resulting from conformational changes in the protein structure; also, enzymatic reactions may be enhanced or retarded by pressure, and their proteins may become more sensitive to enzymatic depolymerization or modification once it has been pressurized. Generally, from the limited studies on the effect of pressure on polysaccharides, it seems that these compounds are not affected by pressure [30].

Mycelia of *Pleurotus* species enable inhibition of growth of pathogenic fungi by using their secondary metabolism products [31], including production of polysaccharides, proteins, enzymes, and triterpenoides [32,33]. The broth of pink species *P. salmoneostramineus* has a higher antifungal activity in comparison to other species broth (**Figure 1**). Bioactivity of *P. salmoneostramineus* may derive from its glycoprotein, called indolone, which plays an important role in the photochemical generation of oxygen from water [28,34].

Also, mycelium ability of species of oyster mushroom differed in its antimicrobial secretions; therefore, the inhibition percentage of *Verticillium* sp. and *T. harzianum* by the mycelia of *P. salmoneostramineus* and *P. cornucopiae* was changeable. This ability is due to the enzymes of *T. harzianum*, which analysed the cell walls of other fungi, thus leading to the stopping of growth of oyster mushrooms over *T. harzianum* [35]. However, the influence of the yellow and pink strains might be due to higher total phenol contents [36].

Angelini *et al*. [11] found that, when essential oils of tea tree *Melaleuca alternifolica* were added to the culture medium, the antagonistic activity of *T. harzianum* against *Pleurotus* spp. was weak. The difference in growth averages of *Trichoderma* sp. was useful to know the contact time between the *Trichoderma* sp. and oyster mushrooms.

The repulsion "deadlock" takes place between different fungal colonies through the pairing on agar, which shows cellular and physical destruction on both sides. Mycelial interference leads to the stoppage of pathogenic fungi growth after swelling cellular and changing the penetration of membranes [37].

However, oyster mushrooms may be a new source of useful bioactive compounds. The ability of oyster mushrooms to produce such metabolic enzymes allows the inhibition of the decay of the cellular walls of pathogenic fungi [37]. Some *Pleurotus* species have shown a disability in overgrowing *T. harzianum.* This could be due to hyphae of this pathogen, which causes cell wall lysis at the point of interaction with *Pleurotus* spp. in the Petri dish [35].

Conclusions

This study investigated the antifungal activities of 4 *Pleurotus* spp. against 3 pathogenic fungi: *Trichoderma harzianum*, *Verticillium* sp., and *Pythium* sp. The anti-fungal activity of the mycelia of oyster mushrooms was tested on PDA using dual culture testing. The antifungal activities of the culture filtrate of oyster mushrooms were tested in liquid and solid media. The highest inhibition activity of 55.56 % was observed with *P. salmoneostramineus* against *Verticillium* sp. *Pleurotus ostreatus* grew over the mycelia of *Pythium* sp. by 5.33 days. The highest inhibition was 12.33 % with *P. salmoneostramineus* against *Verticillium* sp. In liquid media, the highest inhibition was 55 % with *P. ostreatus* culture filtrate against *T. harzianum*.

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