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IN VITRO STUDIES TO DETERMINE ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF THREE *PLEUROTUS* SPECIES (OYSTER MUSHROOM)

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Abstract

Oyster mushrooms (*Pleurotus* spp.) are edible, nutritious and have antifungal and antibacterial potential. *In vitro* antibacterial and antifungal activities of three *Pleurotus* species; i.e. *P. ostreatus*, *P. florida* and *P. eryngii* were examined against five pathogenic fungi including *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum*, *Monilinia fructicola* and five food pathogenic bacteria i.e. *Bacillus subtilis, B. cereus, Escherichia coli, Staphylococcus aureus,* and *Vibrio parahaemolyticus*. The antagonistic activity of oyster mushroom fungal strains was tested by using dual culture method. Amongst all *Pleurotus* species, *P. ostreatus* showed maximum whereas *P. eryngii* possessed minimum antifungal and antibacterial activities. The highest inhibition antimicrobial activity of 90.86% and 92.67% was observed for *P. ostreatus* against *A. alternata* and *E. coli* whereas the minimum 32.33% and 35.9% was recorded for *A. flavus* and *V. parahaemolyticus* for *P. eryngii* respectively. Selected Strains of oyster mushrooms have great potential to replace in-use fungicides.

Key words: Oyster mushroom/ *Pleurotus* spp. antimicrobial activity, Dual culture, Inhibition, Pathogenic fungi, Foodborne bacteria.

Introduction

Oyster mushrooms (genus *Pleurotus*) are nutritious, safe for consumption, ranked second among economically important mushrooms worldwide and contain essential bioactive compounds. The anti-cancer, viral, fungal, bacterial and tumor properties of oyster mushrooms adversely affect pathogenic microbes (Paul*et al*., 2017; Risan *et al*., 2017). Approximately 70 species of *Pleurotus* have been identified. Some oyster mushroom species such as *P. ostreatus*, *P. florida* and *P. eryngii* and *P. sajor-caju* have piqued the attention of researchers in the search for pharmacological metabolites, and are being used as medicines in various parts of the world for ancient times to treat a variety of ailments (Jayakumar *et al*., 2009; Akyuz & Kirbag *et al*., 2009; Kalaw & Albinto *et al*., 2014; Mohamed & Farghaly, 2014). In another research Dawood *et al*., (2021) confirmed the antifungal activity of *P. eryngii* toward the dermatophyte fungus *Trichophyton rubrum*. Gurusamy & Raju (2021) tested synthesized iron nanoparticles of *P. florida* for antimicrobial properties and revealed a stronger inhibitory zone against *E. coli, P. aeruginosa*, *Candida sp., B. cereus*, *S. aureus, Klebsiella pneumoniae, K. terrigena* and *Micrococcus mucilaginosus*.

According to Asri *et al*., (2019), the antimicrobial qualities of oyster mushrooms could be a useful replacement to widely accessible antibacterial and antifungal prescription drugs. Capsules, tablets, and extracts derived from mycelia or fruiting bodies of mushrooms are consumed. Reis *et al*., (2011) and Pérez *et al*., (2020) stated that *P. ostreatus* was one of the most common mushrooms in the world for its high nutritional content, including antioxidant and antibacterial potential. The *P. ostreatus* is already used in bioremediation methods since it absorbs toxic substances (cobalt, copper, iron, and manganese) from polluted soil and water due to possible mineral bioaccumulation in their mycelium and for its beneficial biocompatibility and metal bond formation, including its tolerance to extreme environments, depending on the species, different pH, humidity and temperature requirements (Vamanu, 2012; Kapahi & Sachdeva, 2017).

Many studies have found that due to the wide range of available foods, food-borne microorganisms are common. The most prevalent foodborne bacteria include *E. coli*, *B. cereus*, *P. aeruginosa, Salmonella* spp., *S. aureus*, *Listeria monocytogenes* and *V. parahaemolyticus* are the most common bacteria that cause infectious diseases, food poisoning and digestive disturbance and are resistant to various antibiotics (Jafari *et al*., 2020; Zhang *et al*., 2020). According to Luna-Guevara *et al*., (2019) uremic syndrome, diarrhea, hemorrhagic colitis and other symptoms are caused by *E. coli* in humans and also infect vegetables at any time from pre-harvest to post-harvest during their life cycle. According to Borchers *et al*., (2004), the highest concentration (75%) of chloroform, ethanol, and acetone mushroom extracts of *P. ostreatus* exhibited the greatest inhibition in *S. aureus* and *E. coli* development. Onuegbu *et al*., (2017) proved antibacterial activities of the oyster mushroom extracts towards *Agrobacterium*, *B. cereus*, *P. aeruginosa*, *Streptococcus agalactiae*, and *E. coli* were tested by agar well diffusion method. The results showed that zones of inhibition were maximum in *P. ostreatus* and *P. sajor-caju* for all tested pathogens compared to other oyster species. In another study, Kunjadia *et al*., (2014) proved that the extract of *P. ostreatus* crushed the mycelial colony growth of *Penicillium*, *Mucor luteus*, and *Aspergillus*, with inhibitions percentage of 82, 63 and 78%, respectively. Owaid *et al*., (2017) investigated the antifungal activities of four *Pleurotus* spp. against *Trichoderma harzianum*, *Pythium* sp. and *Verticillium* sp. by dual culture method. The highest inhibition 55% was observed for *P. ostreatus* towards *T. harzianum*.

Oyster mushroom is a highly valued mushroom with significant antimicrobial potential, not only for its nutrient properties, but also because of its strong antibacterial, antifungal, and bioactive compounds that have a beneficial impact on human health and can be considered as a medicinal mushroom. Our results may also confirm using *Pleurotus* strains as an alternative source for the antibacterial and antifungal agent. Therefore, the present work was aimed to evaluate the antifungal and antibacterial potential of different strains of oyster species against several microorganisms under *In vitro* conditions.

Material and Methods

Collection of Oyster culture for antimicrobial bioassay: Three Oyster mushrooms mycelial culture of *P. ostreatus, P. florida and P. eryngii* collected from PMNH (Pakistan Museum Natural History, Islamabad) were investigated in the current study. The received material was sub-cultured on potato dextrose agar (PDA) medium and stored at 25±1°C in Plant Pathology Lab, FQSRI, PARC-SARC, Karachi.

Culture of Bacteria: Five food-borne pathogen bacterial strains *Bacillus subtilis, B. cereus, Escherichia coli, Staphylococcus aureus,* and *Vibrio parahaemolyticus* were used in this study. The bacteria were obtained from the Microbiology Laboratory of the Faculty of Science, University of Karachi. Each bacterial strain was grown from stock culture by streaking them on nutrient agar (NA) and incubated at 37ºC for 16 to 18 hours (overnight). Then, a single colony of each bacterium was cultured overnight in Mueller-Hinton broth (MHB) at 37ºC.

Culture of fungi: Mycelial culture of *Pleurotus* species were selected as a bio-control agent to evaluate their antimicrobial activity against different sequestered phytopathogens. Five pathogenic fungal strains used during the study were *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum* and *Monilinia fructicola* collected from the Plant Pathogen Laboratory of the Food Quality and Safety Research Institute, PARC-SARC, Karachi.

Screening of culture of oyster species for antibacterial and antifungal assay: Dual culture technique was used for checking the antibacterial and antifungal activities of three *Plerotus* species of oyster mushroom. The agar disc of 5 mm of each pathogen from pure culture was inoculated at the periphery of the PDA plates 1 cm away from the edge of the plate, moreover same sized disc of the antagonist was placed opposite to the different pathogen inoculated plates. In the same way, for each pathogen separately, an agar disc containing test pathogens was placed near the edge of a fresh PDA plate and labeled as control. Plates were kept for 7 days at 28 ±1°C to study the interaction of the antagonist with each pathogen.

The interaction was investigated by growing colonies of antagonist and pathogen towards each other. The diameter of each colony with a control plate was measured in centimeters. The percentage of pathogens inhibited by the antagonist was estimated using the formula below (Reddy & Hynes, 1993).

$$\text{Percent inhibition (\%)} = \frac{R1 - R2}{R1} \times 100$$

where; R1 was symbolized as the radius of the pathogen from the control plate and R2 was the radius of the treated pathogen with the antagonist.

Statistical analysis

The data obtained were analyzed by using Analysis of variance (ANOVA) using the Statistical Package for IBM Social Sciences (SPSS), version 20.0 using results presented as Mean ± Standard error and differences were considered significant at $p<0.05$.

Results

The present study showed that the mycelial growth of the phytopathogenic fungal species and food-borne bacterial species were significantly and differentially suppressed by the three strains of oyster mushroom were *P. ostreatus*, *P. florida* and *P. eryngii* (Table 1).

Table 1. Mycelial growth of *Pleurotus* spp. against different pathogenic fungi and bacteria (cm ± S.E) by dual culture method.

Pathogens		*P. ostreatus*	*P. florida*	*P. eryngii*
Bacterial species	*E.coli*	4.16 ± 0.03	4.06 ± 0.07	3.63 ± 0.13
	S.aureus	3.63 ± 0.03	3.33 ± 0.18	3.16 ± 0.12
	B.cereus	2.73 ± 0.12	2.6 ± 0.10	2.4 ± 0.15
	B.subtilis	2.53 ± 0.12	2.36 ± 0.12	2.2 ± 0.23
	V. parahaemolyticus	1.83 ± 0.03	2.73 ± 0.09	1.6 ± 0.25
Fungal species	*A. alternata*	4.06 ± 0.03	3.9 ± 0.10	3.63 ± 0.19
	F. oxysporum	3.83 ± 0.07	3.63 ± 0.18	3.4 ± 0.23
	C. lunata	3.63 ± 0.17	3.46 ± 0.17	3.16 ± 0.09
	M. fructicola	2.73 ± 0.03	2.56 ± 0.19	2.46 ± 0.03
	A. flavus	1.6 ± 0.06	1.83 ± 0.07	1.46 ± 0.18

Antibacterial activity: The result indicated that *P. ostreatus* was found highly effective among all other tested strains and inhibited (92.67%) bacterial growth of *E. coli* whereas least effective (40.79%) for *V. parahaemolyticus* (Figs. 1&2). In the case of *P. florida*, recorded significant antibacterial activity towards *E.coli* (90.34%) on the other hand *S. aureus* (52.48%) showed as the least susceptible bacteria. The strain of *P. eryngii* proved the highest (80%) antagonistic properties against *E.coli* and lowest (35.09%) for *V. parahaemolyticus*. It was also observed that *P. ostreatus* could suppress the mycelial colony growth of all tested bacteria (Fig. 2a-f) whereas, *P. florida* showed more potential *V. parahaemolyticus* (0.78%) (Fig. 1).

Antifungal activity: In the present study, it was observed that the highest fungal biocontrol (90.86) was achieved with *P. ostreatus* towards *A. alternata* and the least (35.33) was for *A. flavus* (Fig. 3). The other strain of *P. florida* showed maximum inhibition percent (86.54) on the growth of *A. alternata* while, minimum percent (40.76) was for *A. flavus* (Fig. 3). More or less same results were obtained for *P. eryngii*, which exhibited the highest inhibition of 80.12 % against *A. alternata* whereas lowest (32.72%) for *A. flavus*. In this study, it is also noticed that *F. oxysporum* was also inhibited by tested *Pleurotus*species, *P. ostreatus*, *P. florida* and *P. eryngii* at 85.69%, 80.38 %, and 75.27% respectively (Fig. 3).

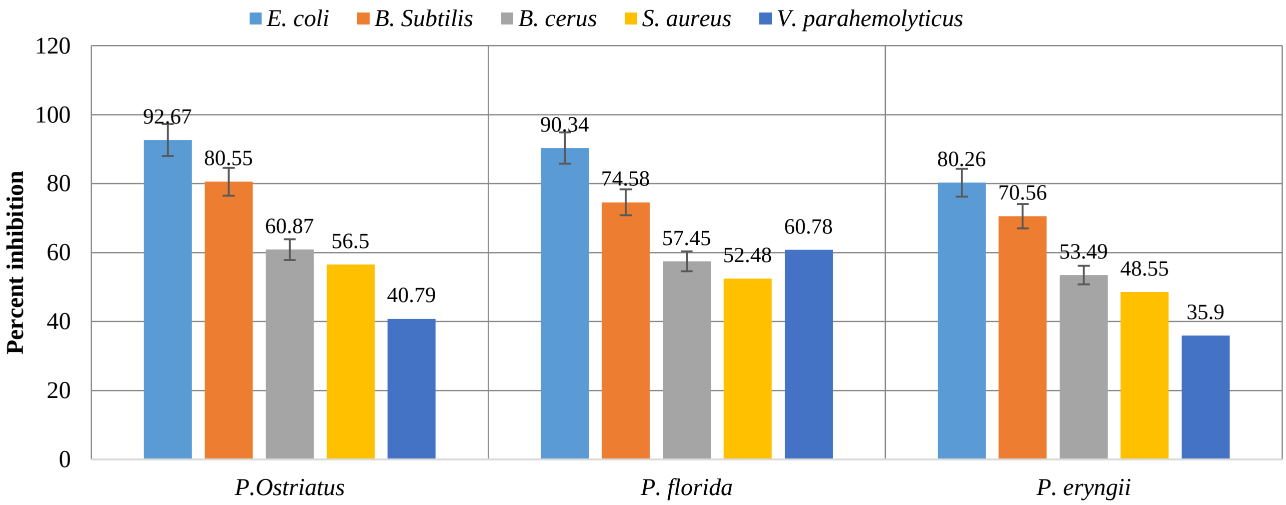

Fig. 1. Percent inhibition of *Pleurotus species* against pathogenic bacteria.

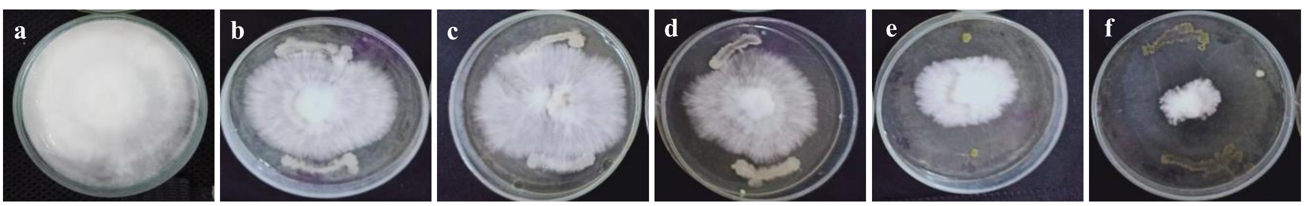

Fig. 2. Antibacterial activity of *P. ostreatus* against foodborne bacteria.
a = Control (*P. ostreatus*), **b** = *E.coli*, **c** = *S. aureus*, **d** = *B. cereus*, **e** = *B. subtilis*, **f** = *V. parahaemolyticus*

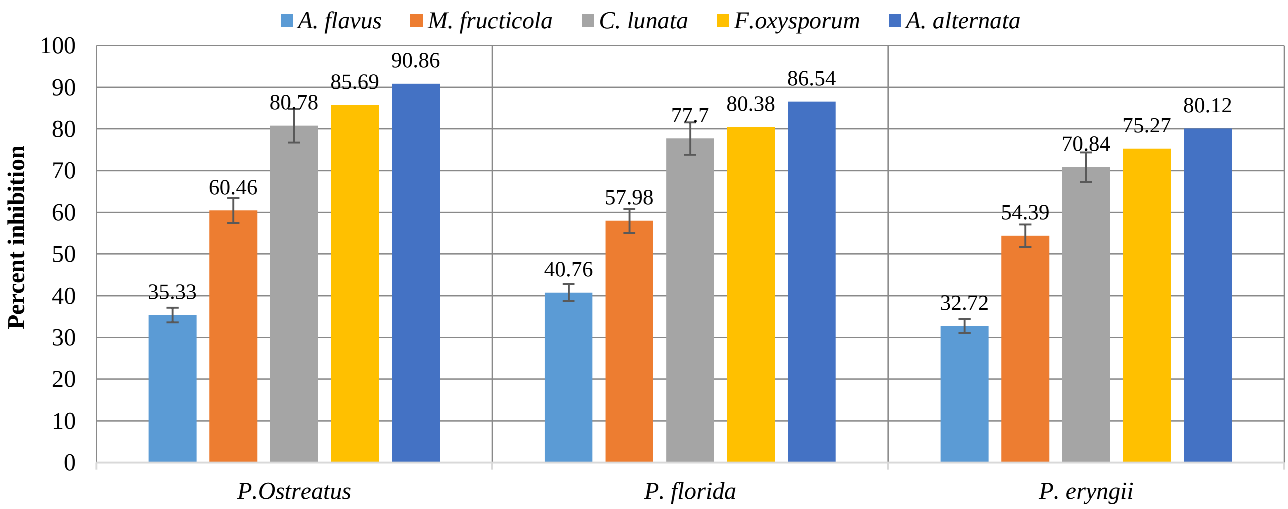

Fig. 3. Percent inhibition of *Pleurotus* species against pathogenic fungi.

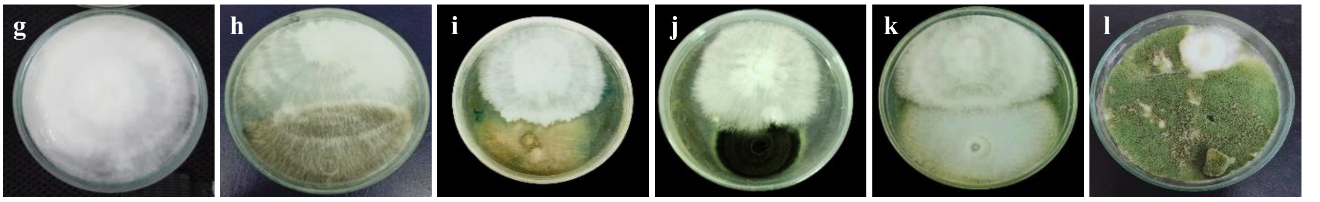

Fig. 4. Antifungal activity of *P. ostreatus* against foodborne bacteria.
g = Control (*P. ostreatus*), **h** = *A. alternata*, **i** = *F. oxysporum*, **j** = *C. lunata*, **k** = *M. fructicola*, **l** = *A. flavus*

In this research, it was also found that the mycelial growth of *C. lunata* was significantly suppressed by *P. ostreatus* compared to *P. florida* and *P. eryngii*. It was also noticed that another pathogenic fungi *A. flavus* was controlled by *P. florida* in contrast to *P. ostreatus* and *P. eryngii*. It was observed that *P. ostreatus* gave the highest inhibition against all tested fungi but was not effective for *A. flavus* (Fig. 4g-l). All three strains of mushrooms could be used to control *A. alternata*, *F. oxysporum* and *C. lunata* while, moderate activity showed towards *M. fructicola* and least was observed for *A. flavus*.

Discussion

Since last couple of decades, the capacity of pathogenic bacteria and fungi to develop resistance against numerous antimicrobial drugs has significantly increased due to the random use of multiple antibiotic medications which are commonly used to treat human illnesses. For this reason, different types of research have recently been conducted to find new sources for the control of pathogenic microorganisms. In the current research amongst all *Pleurotus* species, *P. ostreatus* showed the highest antibacterial potential against *E. coli* and *S. aureus* while moderate activity was recorded toward *B. cereus* and *B. subtilis* whereas, least effective for *V. parahaemolyticus.* Pandey *et al*., (2021) investigated the antibacterial properties of mycelial culture of *P. flabellatus* towards *S. aureus, P. aeruginosa, Shigella flexeneri* and *Proteus vulgaris*. In another study Kovath *et al*., (2021) discovered significant potential of oyster mushroom extract against *E. coli* and *S. aureus*. According to Rathod *et al*., (2021) bioactive elements of numerous *Pleurotus* sp. have exhibited antiviral, antibacterial, antifungal and antimicrobial properties.

This finding was consistent with the findings of (Beltran *et al*., 1997), who found that volatile substances released by the oyster mushroom (*P. ostreatus*) exhibited potent antibacterial properties. *E. coli* were isolated from a variety of fresh fruits and vegetables; although the occurrence was relatively moderate, cause disease in consumers (Zhang *et al*., 2018). Risan *et al*., (2017) proved that chloroform extract of *P. ostreatus* showed maximum percent inhibition against *S. aureus* compared to *E. coli* furthermore *P. ostreatus* was also found more effective than *Agaricus bisporus* against both bacterial species.

In the present study, it was observed that *P. florida* exhibited the greatest inhibition for *E.coli* while remained moderate effective for *S. aureus* whereas the lowest percent inhibition was recorded for *B. cereus* and *B. subtilis*. Our results were well in agreement with the findings of Thillaimaharani *et al*., (2013) who confirmed the antibacterial and antifungal effect of *P. florida* against *E. coli*, *Klebsiella pneumonia*, *K. aoxytoca, Salmonella typhi*, *Vibrio parahaemolyticus*, *V. cholera*, , *Proteus murabilus* and *Streptococcus* sp. and the fungal species *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Microsporum gypseum*. In the case of *Plerotus eryngii*, the highest percent inhibition was recorded for *E. coli* during the current study whereas it showed moderate antibacterial activity against *S. aureus* while it did not control three food poisoning bacterial species, *B. cereus*, *B. subtilis* and *V. parahaemolyticus*. According to Gashaw *et al*., (2020), the methanolic extracts of *P. florida* and *P. ostreatus* were recorded for the highest antibacterial activity against *E. coli*. Bawadekji *et al*., (2017) demonstrated that crude extract of *P. ostreatus* exhibited significant zone of inhibition against *P. aeruginosa*, *C. albicans*, and *S. aureus* whereas the extract of *P. eryngii* towards four food-borne bacteria: *E. coli*, *S. aureus*, *S. epidermidis*, and *P. aeruginosa*. Interestingly, all of the *Pleurotus* species studied had antibacterial activity. Our findings contradicted those of Chowdhury *et al*., (2015) who found that *P. ostreatus* is the least effective mushroom extract for inhibiting bacterial growth. Venturini *et al*., (2008) reported that *P. ostreatus* displayed no inhibition when tested with *B. cereus*, *V. parahaemolyticus* and *S. aureus*, indicating that these bacteria might be resistant to the mushroom extract while in our study *P. ostreatus* showed a zone of inhibition against *B. cereus* and *S. aureus* whereas did not exhibit against *V. parahaemolyticus*. According to the research *V. parahaemolyticus* is the most common seafood-borne pathogen, which is responsible for the highest prevalence of seafood associated with different infections of ingestion problems in humans (Mok *et al*., 2019; Jafari-Sales *et al*., 2020). In another study, Youssef *et al*., (2008) reported the aqueous extract of *P. ostreatus* had strong inhibition properties against diverse bacterial strains comprising *Mycobacterium aurum*, *S. aureus*, *Streptococcus* sp., *Acinetobacter calcoaceticus* and *Klebsiella* sp., *B. cereus*, *B. subtilis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

The present study revealed that the *S.aureus* which was the second most foodborne bacteria after *E.coli* was controlled by all *Pleurotus* species. According to the literature, *S. aureus* is a major bacterial human pathogen that causes a wide range of clinical symptoms. According to Liu *et al*., (2019) *S. aureus* is ubiquitous in the environment and is found in the mucous membranes and skin of most humans. *S. aureus* can cause a variety of potentially serious illnesses. *S. aureus* contamination in food safety has become a worldwide health issue. *S. aureus* widely exists in the air, water, dust, human and animal excretions, which makes the food much easier to become contaminated; it can produce enterotoxin and increase the risk of food poisoning (Yu *et al*., 2016). It has been reported that in the United States, nearly half a million hospitalizations and 50,000 deaths occur resulting from *S. aureus* each year (Schlecht *et al*., 2015). Our results were in accordance with Asri *et al*., (2019), who found that *S. aureus* was the most susceptible bacteria when being tested with ethanolic extracts of oyster mushroom. In the present study, all oyster mushrooms strain tested exhibited antibacterial activity against *E. coli, S. aureus, B. subtilis* and *B. cereus* except *V. parahaemolyticus.*

In the present study, the strain of *P. ostreatus* was found highly effective against three tested pathogenic fungi *A. alternata*, *F.oxysporum* and *C. lunata* whereas moderate activity was noticed toward *M. fructicola* while was not found to be effective to suppress the mycelial growth of *A. flavus*. The current study showed that all tested strains of oyster mushrooms exhibited the highest antifungal properties against *A. alternata* causing pre and post-harvest diseases in numerous crops and worldwide economic losses. *A. alternata* alone is recorded as causing disease on over 100 host plants including brown leaf spots, stem canker, leaf blight, fruit spot, seed, and root rot in various plants (Kgatle *et al*., 2018; Da Cruz *et al*., 2019; Khan *et al*., 2020; Haque & Parvin, 2021). The present study demonstrated that *F. oxysporum* was the second most plant pathogic fungi inhibited by all tested strains of oyster mushroom. Our results are in accordance with those reported by Chu *et al*., (2005) the aqueous extract of fresh fruiting bodies of the *P. ostreatus* had an inhibitory effect on the mycelial growth of *F. oxysporum* and *A. niger*. *F. oxysporum* is the causative agent of fusarium wilt which has proved to be the most destructive disease affecting a wide range of plants comprising of weeds and commercially domesticated plants and crops. The disease results in varied symptoms ranging from the browning of vascular tissues, yellowing of leaves, plant death and inhibits the growth of the plant. The management of *Fusarium* has been difficult due to its soil-borne nature (Flood, 2006; Joshi, 2018).

In the current study, it was also discovered that of all the fungal species tested, *A. flavus* was shown to be only difficult to suppress by all *Pleurotus* species. Our findings were in line with those of Kumar and Yadav (2014) who investigated the antifungal potential of *P. ostreatus* against 6 fungi, including *A. flavus*, *A. fumigatus*, *P. chrysogenum*, *Sporotricum carnis*, *Thermoascus aurantiacus* and *Humicolagrisea*. *P. chrysogenum* demonstrated the most resistance, while *A. flavus* showed the least. Furthermore, our research did not agree with the results of Hussien *et al*., (2015) who examined that fresh ethanolic extract of *P. ostreatus* hindered the mycelial growth of *A. flavus* significantly, followed by *F. moniliforme* and *P. expansum* respectively. In another study according to Roy *et al*., (2016), ethyl acetate extract of *P*. ostreatus exhibited a modest antibacterial efficacy *In vitro* against 10 bacterial strains while it had no antifungal properties against *A. niger*, *A. orchareus* and *C. albicans*. The analysis of variance demonstrated that the dual culture method at 0.01 level showed a significant difference and its effect on pathogenic fungi and bacteria are highly significantly different for all levels ($p \leq 0.001$).

In our study, *P. ostreatus* was the most potent among all *Pleurotus* strains, due to its high inhibitory effect against most tested bacterial and fungal organisms whereas *P. florida* and *P. eryngii* both had the greatest antifungal activity against *A. alternata* and *F. oxysporum*, the moderate effect was noticed for *C. lunata* and least for two pathogenic fungi *M. fructicola* and *A. flavus*. In the future, the oyster mushroom strain could be used to combat bacterial foodborne and plant pathogenic diseases caused by fungi.

Conclusion

It was concluded from the results of the present work that *P. ostreatus* possess great antifungal and antibacterial activity against all tested pathogens, suggesting that *P. ostreatus* strain could be used as a natural source of the antifungal and antibacterial agents in the treatment of diseases for fungal and bacterial infections in humans and plants instead of the use of commercial antifungal and antibacterial drugs which result in drug or pesticides resistance.

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