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Effect of Different Incubation Times on the Effectiveness of Oyster Mushrooms Towards the Growth of Some Fungal Isolates

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Abstract

The study was conducted by preparing the filtrate of the oyster mushroom Pleurotus ostreatus, where the effectiveness of the fungal filtrate prepared from growing the fungus in the liquid Potato Dextrose Broth (PDB) medium was selected for two different periods (14, 28 days) in inhibiting some types of fungi (Aspergillus Spp : Aspergillus niger ،Aspergillus flavus, Aspergillus terreus) and some types of fungi (Candida Spp: Candida albicans, Candida tropicalis, Candida dubliniensis). Two concentrations of the filtrates taken for two different incubation periods were prepared (the original concentration of the filtration, half concentration of it). The results showed an inhibitory effect of the oyster filtrate from the two different incubation periods on the growth of the fungal isolates used, and that the fungal filtrate prepared from the 28-day period was the most effective in producing the highest inhibitory values, with diameters ranging between 22–45 mm, while the diameters for the inhibition resulting from the effect of the fungal filtrate prepared from the period of 14 days, ranged between 18–28 mm.

Keywords: *Aspergillus niger, Aspergillus flavus, Aspergillus terreus*

INTRODUCTION

Pleurotus mushroom is one of the most important edible oyster mushrooms and all its species are edible and most of them have been produced and grown commercially as it is the third largest cultivated mushroom in the world. It is considered some healthy food rich in proteins and unsaturated fats with low calories, rich in chitin and the B vitamins group, which are Thiamin (B1), Riboflavin (B2), Nicin (B5), Pyridoxine (B6), Biotin (B7). It is rich in minerals and a source of enzymes that break down quinine and phenol [1]. It also has a distinct effect in reducing cholesterol in the blood serum, in addition to its clear effect as an anti-cancer agent and in stimulating cellular immunity [2] along with treating many diseases since ancient times in many countries, especially India, China and Japan, due to its importance in activating the immune system in the human body [3]. This mushroom has a high nutritional value, as it contains a good amount of protein, vitamins, minerals and fibers. Oyster mushrooms also contain active substances of high medical value for human health. Research has shown that these mushrooms activate the body's immune system and act as anti-cancer agents [4], anti-inflammatory agents, cholesterol-lowering agents, heart disease and blood pressure agents. These compounds have been extracted, separated and prepared in the form of tablets, capsules, syrup and injection ampoules in several countries, most notably China and Japan. The medical importance of food fungi has been recognized for thousands of

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years, as Pleurotus mushrooms have been used in the preparation of folk medicines in China and have been used to treat diseases, prolong life, strengthen memory and as sedatives. Species belonging to the genus Oyster mushroom are characterized by a high ability to grow and the speed of mycelial growth on different culture media and production in different environmental conditions [5]. Currently, oyster mushrooms rank second after the white agricultural mushroom Agaricus Bosporus, accounting for 25% of global food fungi production [6]. It is unique in its high nutritional value because it is rich in protein, which constitutes 20–40% of the dry weight [7]. Diseases caused by fungi and yeasts are known as mycoses and are usually chronic infections because fungi grow slowly and are difficult to treat because they are eukaryotic organisms, making them of great medical importance at present. Pathogenic fungi have the ability to cause severe damage to humans due to the secondary metabolites they produce during their growth, which have toxic properties. It has been shown that the damage caused by fungal diseases to humans is mainly due to the enzymes and toxins produced by them [8]. Fungal diseases are often difficult to control because they require specialized antifungals, but the use of antifungals in treatment is not useful as is the case with the use of antibacterials because fungi are eukaryotic, in addition to the lack of vaccines against fungi [9].

The yeast *C. albicansa* is an opportunistic fungus and turns from a symbiotic organism to a pathogen due to its virulence factors, such as the formation of the germ tube, adhesion to epithelial surfaces, and the production of extracellular enzymes, as the yeast works to analyze tissues, penetrate them, and reach the rest of the body [10]. Due to the abundance of research and studies on the nutritional and therapeutic importance of oyster mushrooms, the study aimed to evaluate the possibility of the effect of oyster mushroom filtrate on inhibiting some types of filamentous fungi and yeasts.

METHODOLOGY

Activation and Development of the Oyster Mushroom Isolate

A 7 cm diameter disk of the fungal isolate was transferred using a cork piercer and transferred using a sterile needle to the surface of sterile Petri dishes containing solid potato dextrose medium (PDA). The dishes were then incubated at 27°C for a period ranging from 5–7 days, and the isolate was multiplied by growing it on these nutrient media (Figure 1) [11].

Figure 1. Oyster mushroom *Pleurotus ostreatus* grown on PDA medium.

Activation and Development of Oyster Mushroom Isolate

A 7 mm diameter disc of the original isolate of the fungus was transferred using a cork piercer and transferred using a sterile needle to the surface of sterile Petri dishes containing solid potato dextrose medium (PDA). The dishes were then incubated at 27°C for 5–7 days using a 1000 ml glass flask with a 250 ml medium prepared, and the isolation was propagated by growing it on these nutritional media (Figure 2(a–b)) [12].

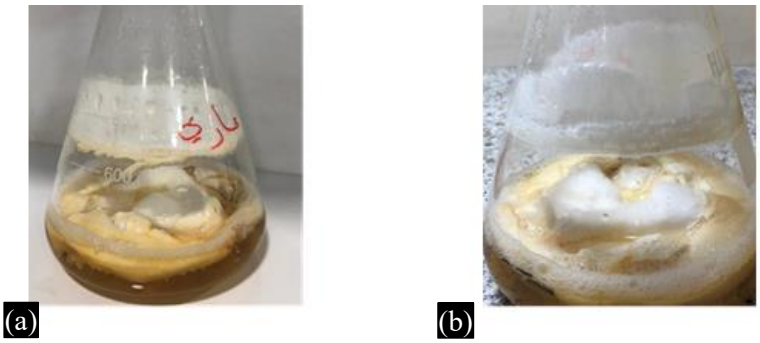

Figure 2. Oyster mushroom *Pleurotus ostreatus* grown in PDA medium during two different incubation periods. (a) Incubation period 28 days, (b) Incubation period 14 days.

Preparation of Oyster Mushroom Filtrate

The study relied on preparing oyster mushroom filtration during two different incubation periods (14 days and 28 days). Two flasks were prepared to grow oyster mushrooms, where the mushroom taken from a culture plate 5–7 days was grown by cutting a disc with a diameter of 9 mm, in a 1000 ml flask with 250 ml of PDA medium [12]. After the incubation period ended, the fungal isolate (fungal biomass) growing in the liquid medium was separated using sterile filter papers Whatman No. 1, and then the filtrate was sterilized using a Millipore Filter System with 0.45 micrometer holes (Figure 3).

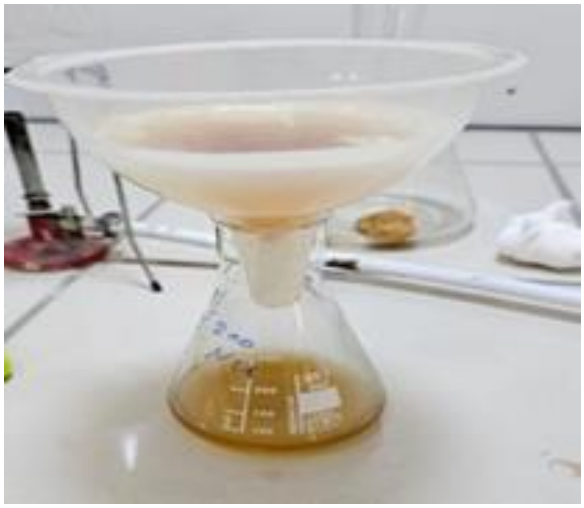

Figure 3. Method of filtration.

Evaluation of the Effectiveness of Oyster Mushroom Filtrate Against the Fungal Isolates

The method included preparing two concentrations of oyster mushroom filtrate prepared from growing the fungus in 14 and 28 days, where two concentrations of the fungal filtrate were used, the original concentration and half of it.

Testing the Effectiveness of Oyster Mushroom in Inhibiting the Growth of Candida Fungal Isolates and Their Types

The diffusion method was used to test the effectiveness of oyster mushroom filtrate against the fungi used in the study by diluting colonies taken from a Petri dish containing SDA medium 24 hours at a temperature of 37°C, where distilled water was used for dilution. They were mixed well using a Vortex vibrator to obtain a homogeneous fungal suspension, where the turbidity ratio was regulated to reach 2.8–3.2 cells/ml using a turbidity meter [13]. 0.2 ml of fungal inoculum was spread over the entire surface of SDA using sterile ear swab and 3 holes were made in each dish using 8 mm diameter cork drill, two holes were filled with 750 µl of the used concentrations of fungal filtrate prepared from growing the fungus for 14 days (original concentration) and half the concentration, while the third hole

was filled with distilled water for control. The method was applied with three replicates. After an incubation period of 24 hours at 37°C, the dishes were examined for the purpose of measuring the diameter of growth inhibition [14]. This method was also applied using fungal filtrate prepared from growing oyster mushrooms for 28 days **(**Figures 4 and 5).

Figure 4. Turbidity tester.

Figure 5. The prepared fungal vaccine from *Candida* isolates.

Testing the Effectiveness of Oyster Mushroom in Inhibiting Some Types of Aspergillus spp. Isolates

The diffusion method in the hole was used to test the effectiveness of the oyster mushroom filtrate used in the study. The fungal inoculum was prepared from the fungal isolates used in the study by diluting colonies taken from a Petri dish containing PDA medium for 5–7 days at a temperature of 27–28°C. Distilled water was used for dilution and mixed well using a Vortex vibrating device to obtain a homogeneous fungal suspension. The turbidity ratio was regulated to reach $8^{10} \times 1.5$ cells/ml using a turbidity test meter [13]. 0.2 of the vaccine was spread over the entire surface of the PDA using a sterile ear swab by planning and 3 holes were made in each dish using a cork drill with a diameter of 8 mm and two holes were filled with 750 microliters of the concentrations used from the fungal filtrate prepared from growing the fungus for 14 days, while the third hole was filled with distilled water for control with three replicates. After an incubation period of 48 hours at a temperature of 27–28°C, then the growth was monitored, and the dishes were examined for the purpose of measuring the diameter of growth inhibition [15]. This method was also applied using the fungal filtration prepared from growing the oyster mushroom for 28 days (Figure 6).

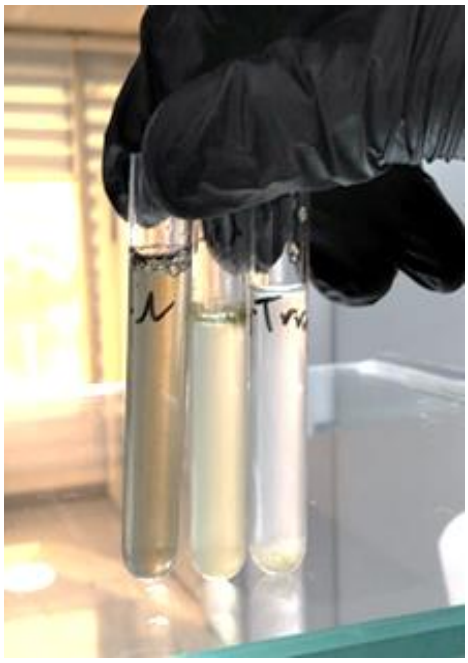

Figure 6. The prepared fungal vaccine from *Aspergillus* isolates.

RESULTS AND DISCUSSION

The results showed an inhibitory effect of the concentrations used from the oyster mushroom filtrate prepared during two different incubation periods 14 and 28 days on the fungal isolates used in the study Aspergillus spp. (*C. albicans, C. tropicalis, C. dubliniensis*) and Candida spp. (*C. albicans*, *C. tropicalis* and *C. dubliniensis*) where the original concentration of the stock filtrate was the most effective in causing the highest inhibition values on the growth of the isolates used in the study and during the two different incubation periods, and the half concentration of the filtrate was the least effective during the two incubation periods on all the isolates used except for the isolate *C. dubliniensis*. The study showed that the filtrate prepared from growing oyster mushrooms for a period of 28 days was the most effective in producing the highest inhibitory values for the isolates used in the study, as the diameters of inhibition for the *Aspergillus spp.* isolates used at the original concentration of the filtrate were 35, 30, 45 mm and at the half concentration they reached 28, 22, 34 mm for each of (*A. niger, A. flavus*, *A. terreus*), respectively, while the diameters of inhibition for the *Candida* spp. isolates used in the study reached at the original concentration of the filtrate 26, 28, 24 mm for the isolates (*C. albicans, C. tropicalis, C. dubliniensis*), respectively, and the half concentration of the filtrate produced inhibitory values of 19, 20, 18 mm for the same isolates, respectively (Tables 1 and 2). The inhibitory effects of Oyster Mushroom filtrate on fungal isolates were observed, with notable differences based on the duration of growth. *Aspergillus* spp. and *Candida* spp. showed inhibition when treated with filtrate from 14-day-old cultures (Figures 7 and 8) and 28-day-old cultures (Figures 9 and 10), respectively.

Table 1. Diameters of inhibition zones in *Aspergillus spp.* isolates and *Candida spp.* Isolates under the influence of two concentrations of oyster mushroom filtrate grown for 14 days.

Inhibition Diameter (mm)		Isolation	No.
Second Conc. (Half Conc.)	***First Conc. (Original)***		
26	32	*A.niger*	1
16	20	*A.flavus*	2
24	30	*A.terreus*	3
14	22	*C.albicans*	4
25	31	*C.tropicalis*	5
12	19	*C.dubliniensis*	6

Table 2. Diameters of inhibition zones in *Aspergillus spp.* isolates and *Candida spp.* isolates under the influence of two concentrations of oyster mushroom filtrate grown for 28 days.

Inhibition Diameter (mm)		Isolation	No.
First Conc. (Original)	*First Conc. (Original)*		
28	35	*A. niger*	1
22	30	*A. flavus*	2
34	45	*A. terreus*	3
19	26	*B. albicans*	4
20	28	*C. tropicalis*	5
18	24	*D. dubliniensis*	6

A.niger *A.terreus* *A.flavus*

Figure 7. Inhibitory diameters of *Aspergillus spp.* fungus isolates by the effect of oyster mushroom filtrate prepared by growing oyster mushroom for 14 days.

C.albicans *C.tropicalis* *C.dubliniensis*

Figure 8. Inhibitory diameters of *Candida spp*. fungal isolates by the effect of oyster mushroom filtrate prepared by growing oyster mushroom for 14 days.

A.niger *A.terreus* *A.flavus*

Figure 9. Inhibitory diameters of *Aspergillus spp.* fungus isolates by the effect of oyster mushroom filtrate prepared by growing oyster mushroom for 28 days.

The results showed an inhibitory effect of the oyster mushroom filtrate on the fungal isolates used in the study, and that this effect can be attributed to the various active compounds produced by the fungus

as secondary metabolites, the most important of which are flavonoids and antioxidants, which were found to increase after 8–60 days of growth [16, 17]. Increased growth was observed after 12 days of mushroom growth [18]. Many studies have indicated the antimicrobial effectiveness of oyster mushrooms against a group of microorganisms and fungi, as its filtrate contains the compound p-anisaldehyde, which has an inhibitory property for some fungi, including *Aspergillus niger* [19, 20]. The sensitivity of the *A. niger* fungus was greater towards the inhibitory action than *A. flavus* [21, 22]. Despite the various compounds produced by Oyster mushrooms, the largest amount of tannin produced compared to other compounds [23] after 12 days of mushroom growth [24, 25]. The increase in its production may be associated with the high inhibitory capacity of the fungal filtrate towards the isolates used in the study after 28 days of oyster mushroom growth compared to its inhibitory capacity after 14 days [26].

Figure 10. Inhibitory diameters of *Candida spp*. fungal isolates by the effect of oyster mushroom filtrate prepared by growing oyster mushroom for 28 days.

Recommendations

1. Testing the antioxidant effectiveness of oyster mushroom filtrate.
2. Detection of active compounds produced by oyster mushroom.
3. Testing the effectiveness of oyster mushroom filtrate against some types of bacteria.
4. Studying the effect of different conditions on the secondary metabolites of the fungus and testing its biological effectiveness, such as temperature, pH, and culture media used for development.

CONCLUSIONS

The study exhibited that the remainder prepared from growing oyster mushrooms for a period of 28 days was the most effective in producing the highest inhibitory values for the isolates used in the study, as the diameters of inhibition for the *Aspergillus spp.* isolates used at the original concentration of the filtrate were 35, 30, 45 mm and at the half concentration, they reached 28, 22, 34 mm for each of (A. *niger, A. flavus*, *A. terreus*), respectively.

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