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Evaluation Effect Of Pleurotus Ostreatus On Some Virulence Factors In Aspergillus spp

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Evaluation effect of *Pleurotus ostreatus* on some virulence factors in *Aspergillus spp*

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ABSTRACT

This study include isolation some types of *Aspergillus sp* from different sources such as (soil, some vegetables), three species appeared on medium sabroud dextrose agar ; *Aspergillus fumegatus* , *Aspergillus nidulans and Aspergillus ochracaeus* ,then detected the virulence factors of these fungi include production of these enzymes (proteinase ,phospholipase ,heamolycin ,lipase and urease) , the three species give positive result for production of phospholipase ,heamolycin ,lipase and urease; while proteinase product only from *A. ochracaeus* . Also tested the inhibitory effect of the *Pleurotus ostreatus* filter in three concentration 10%, 20% and 30% on the radial growth of the fungi under study, the *Pleurotus ostreatus* filter inhibit the radial growth and the radial growth reached 19.5mm , 48.8mm and 19.5mm for the fungi *Aspergillus fumegatus* , *Aspergillus nidulans and Aspergillus ochracaeus* respectively , and also in this study detect some antifungal drugs (Fluconazole , miconazole and nystatin) in concentration 20% on the radial growth of these fungi and found that antifungal drugs have high inhibitory effect on growth .

1. Introduction

All type convert to species of *Aspergillus sp* are fungi that exist in soil , dust and decomposed organic materials , also composed 25% from air born fungi (3) . Also they found on vegetables and fruits , they are opportunistic fungi , cause Aspergillosis and fungus ball (aspergilloma) (15) . The types of *Aspergillus sp* have virulence factors that enhance growth and survival in lungs after weakness of person immunity as result of diseases and used of immunosuppressant treatments (18) . The virulence factors of these fungi production of toxins and enzymes (27), as well they have other virulence factors such as the ability of growth in different temperatures , growth in host pH and small size of conidia (10). Also they have the ability for adhesion and production of enzymes (proteinase ,phospholipase ,heamolycin ,lipase and urease) (29). The *Pleurotus ostreatus* is from basidiomycetes , it has high nutritional value so contain high rate of protein (6), also it contains carbohydrates, vitamins and unsaturated fats; also has immune system stimulants (21,8). This fungi has inhibitory importance for pathogenic fungi , studies have confirmed its inhibitory importance for pathogenic fungi , bacteria , viruses and decomposed of mycotoxins (8).It also contains compounds (1- octen -3 – l) it is anti-bacterial (17). The *Pleurotus ostreatus* has acceptable taste and smell and saved by drying , canning and freezing (20) , it was contain active defense materials for body that used as anti-toxin and anti-inflammatory (14), also contain Lectin that raises the immune response for lymphocyte and macrophage (22)

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2. Methodology

2.1.Detection of virulence factors

Tested these fungi(*Aspergillus fumegatus , Aspergillus nidulans and Aspergillus ochracaeus*) about virulence factors by production of enzymes :

A -proteinase enzyme production : by culture fungi on skimmed milk medium which prepared by dissolved 20 gm of agar in 900 ml distilled water in flask , and in another flask dissolved 10 gm of skimmed milk powder in 100 ml distilled water , the two solutions sterilized in auto cleave each one separately sterilized and after cool to 45º, the two solution mixed together and add the antibiotic chloramphenicol , then poured in plates , after solidified culture each type of fungi under study in the center of plates by used cork piercing , the plates incubated in incubator in 28º for 72 hours . (1)

B - lipase enzyme production : by culture fungi on peptone Tween 80 , which prepared by dissolved 10 gm. of peptone , 20 gm agar , 0.1 gm hydrated calcium chloride and 5 gm of sodium chloride in 1 liter of distilled water in flask and sterilized in auto cleave ,after cool the medium to 40 º add 10 ml of Tween 80 and chloramphenicol , then poured in plates , after solidified culture each type of fungi under study in the center of plates by used cork piercing , the plates incubated in incubator in 28º for 72 hours (28).

C -urease enzyme production : by culture fungi on urea agar medium which prepared by dissolved 2.4 gm of urea agar base in 95 ml distilled water , sterilized in auto cleave and then cool to 45 º and then add 5 ml of urea solution sterilized by Millipore filter , add the antibiotic chloramphenicol , then poured in plates , after solidified culture each type of fungi under study in the center of plates by used cork piercing , the plates incubated in incubator in 28º for 72 hours (12).

D -heamolycin enzyme production : by culture fungi on blood agar medium , which prepared by dissolved 28 gm of blood agar medium in 1000 ml distilled water sterilized in auto cleave and then cool to 45 º and then add blood sheep in the rate 5% add the antibiotic chloramphenicol , then poured in plates , after solidified culture each type of fungi under study in the center of plates by used cork piercing , the plates incubated in incubator in 28º for 72 hours (19).

E- phospholipase enzyme production : by culture fungi on Egg Yolk agar medium , this medium prepared by add 1 molar of NaCl and 0.005 molar of CaCl2 to 1 liter of sabroud dextrose agar , sterilized in auto cleave , after cool to 45º add sterilized egg yolk powder in the rate 8% add the antibiotic chloramphenicol , then poured in plates , after solidified culture each type of fungi under study in the center of plates by used cork piercing , the plates incubated in incubator in 28º for 72 hours (30) . .

2.2. preparation of the *Pleurotus ostreatus* filter : prepared by grow fungus in flask (250 ml capacity) contain PDB (potato dextrose broth) for three weeks with shake every 2-3 days , then the fungus growth filtered with Whatman no1 and the fungus filter sterilize with Millipore filter 0.22mm (4).

2.3. tested the effect of the *Pleurotus ostreatus* filter on pathogenic fungi in PDA medium :

Used food poisoning technique to find out the effect of the *Pleurotus ostreatus* filter on radial growth for pathogenic fungi (9) , the filter prepare in 10%, 20% and 30% concentration of PDA medium , after poured the medium and medium solidified has been put 5 mm of each fungal growth in center of plate by using cork piercing , all plates incubated in incubator in 25º for 7 days (4).

2.4. Tested the effect of antifungal drugs on growth of pathogenic fungi :

Used food poisoning technique to find out the effect of the antifungal drugs (miconazole , fluconazole , nystatin) on radial growth of pathogenic fungi by added the drugs to the PDA medium in concentration 20%. (4).

3.Results & Discussion

3.1. Virulence factors of fungi:

Results shows figure(1 :A,B,C) that all of *Aspergillus sp* under study produce lipase enzymes and it was revealed by found of white precipitate around the colony ; also all fungi under study produce urease and it was revealed by change the color of medium from yellow to red because the analyzing of urea in medium and released of ammonia that converted the medium to basic ; as well phospholipase enzyme produce from all fungi under study and it was revealed by found of white precipitate around the colony on Tween 80 medium , the heamolycin produce from all fungi under study and it was revealed by decomposed of blood on blood agar medium ; the proteinase enzyme produce only from *A. ochraceus* that form a white aura around the colony**.**

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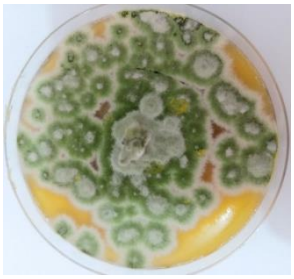
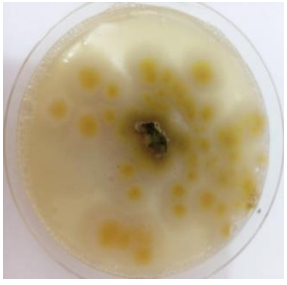
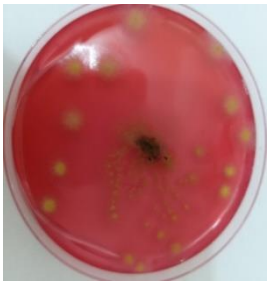


a-Phosphplipase



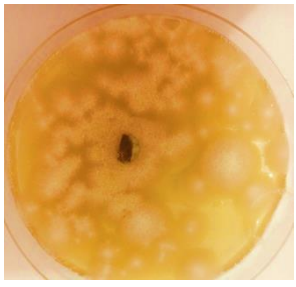
b- lipase



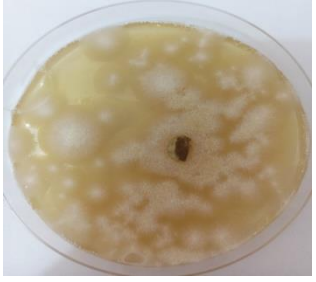
c- urease

d- heamolycin

A- virulence enzymes of *Aspergillus .nidulance*



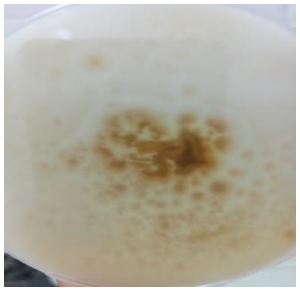
a-Phosphplipase



b- lipase

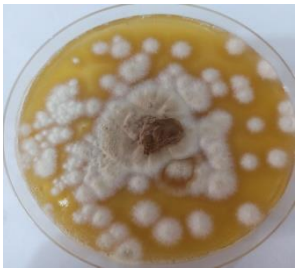
c- urease

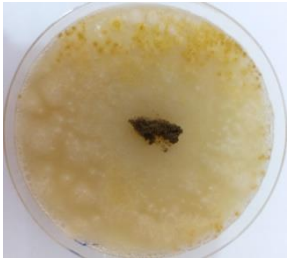
d- heamolycin



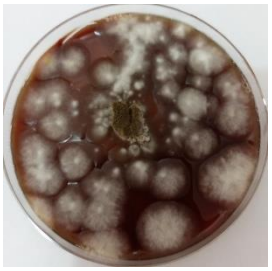
c-proteinase

(B- virulence factors of *Aspergillus ochraceaus*)


a-Phosphplipase


b- lipase


c- urease


d- heamolycin

C-virulence factors of *Aspergillus fumegatus*

Figure 1 (A,B,C) virulence factors of pathogenic fungi.

The ability of fungi for production virulence enzymes like proteinase , lipase , phospholipase , urease and heamolycin increase the pathogenicity of fungi towards host (5). The production of lipase enzyme make the fungi to growth on organic materials and as it composed fatty acids and used as asource of carbon (23).

Production of phospholipase it is one of the most important virulence factors because it analyze the vital components of the cell membrane of the host and lead to weak the membrane and disrupting its work , that because membrane contain lipids and proteins(13)

These results agree with (2 , 3) who found the types of *Aspergillus sp* superior in hemolysis compared to other fungi , also results agree with (26) who tested production of virulence enzymes such as proteinase , lipase , phospholipase and heamolycin from some types of *Aspergillus sp* , he found it to be product for these enzymes at high rate .

3.2. The effect of the *Pleurotus ostreatus* filter on growth of pathogenic fungi :

The results show that the *Pleurotus ostreatus* filter inhibited the radial growth of these fungi in different concentrations , the rate of radial growth of *Aspergillus fumegatus* , *Aspergillus nidulance* and *Aspergillus ochraceaus* was 29.3 mm , 68.4 mm and 34.2 mm respectively in Concentration of *Pleurotus ostreatus* filter 10% .and in concentration 20 % the rate of radial growth was 29.3 mm , 68.4 mm and 20.5 mm for the fungi *Aspergillus fumegatus* , *Aspergillus nidulance* and *Aspergillus ochraceaus* respectively . the rate of radial growth of *Aspergillus fumegatus* , *Aspergillus nidulance* and *Aspergillus ochraceaus* in concentration 30 % of *Pleurotus ostreatus* filter was 19.5 mm , 48.8 mm and 19.5 mm in comparsion with the control 90 mm in all fungi table (1) which shows that there is no significant differences in at the level of probability 5% .

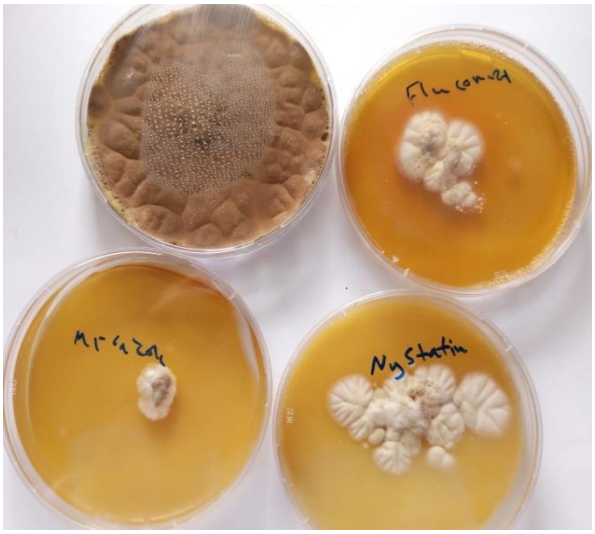
Table (1) The effect of the *Pleurotus ostreatus* filter on growth of pathogenic fungi

The name of fungi	**Radial growth rate measured in millimeter**			
		Concentration of *Pleurotus ostreatus* filter		
	control	10%	20%	30%
Aspergillus fumegatus	90	29.3	29.3	19.5
Aspergillus nidulance	90	68.4	68.4	48.8
Aspergillus ochraceus	90	34.2	20.5	19.5
L S D 0.05	2.29	0.12	0.11	0.08

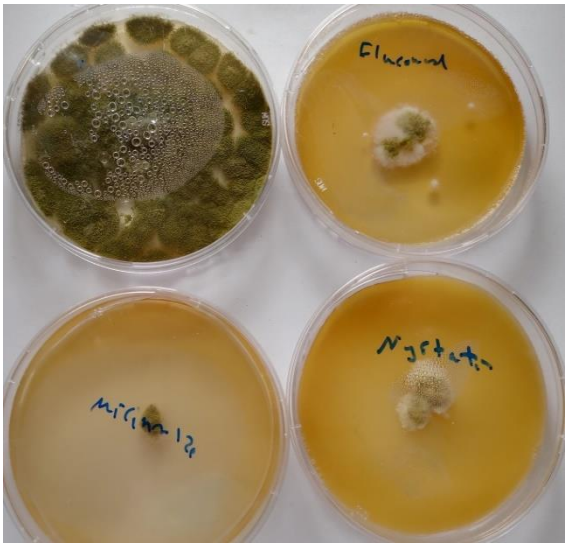
These results agree with (16) who found that the *Pleurotus ostreatus* filter contain active compounds and enzymes have active role in inhibit the growth of pathogenic fungi under study , also results agree with (7, 31) that they found the *Pleurotus ostreatus* contain anti –bacterial and anti-microbial materials .

3.3. **The effect of antifungal drugs on pathogenic fungi** :

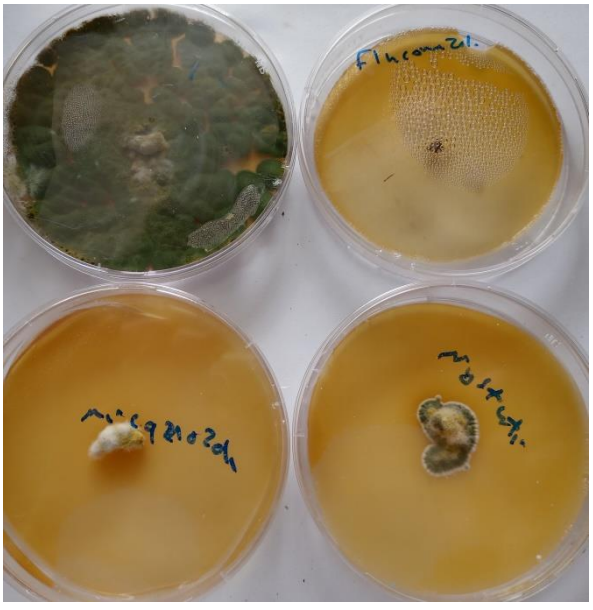
The results show that the antifungal miconazole has the most inhibitory effect on the growth of fungi *Aspergillus ochraceaus* and , *A. fumegates* ,while the antifungal fluconazole has the most inhibitory effect on the growth of fungi *A. nidulance* ,the effect of antifungal drugs explained in figure (2) .



Aspergillus ochraceaus



.Aspergillus. fumegates



Aspergillus . nidulance

Figure 2 the effect of antifungal drugs on radial growth of fungi under study

These results agree with (11) who tested the effect of antifungal drugs on three types of *Aspergillus sp* , he found that they have inhibitory effect on *Aspergillus . nidulance*

and . *Aspergillus. fumegates* , also results agree with (24) who found inhibitory effect of antifungal drugs on growth of *Aspergillus sp*, results also agree with (25) who found that antifungal drugs have inhibitory effect in low concentrations and killer effect in high concentrations

Conclusion:

1-Some types of *Aspergillus sp* contains virulence factors such as secretion of enzymes such proteinase , lipase , phospholipase , urease and hemolycin.

2- *Pleurotus ostreatus* has inhibitory effect on radial growth of pathogenic fungi .

3- The antifungal drugs (Fluconazole , miconazole and nystatin) in concentration 20% inhibit the radial growth of pathogenic fungi under study .

Conflict of Interest: The authors declare that they have no conflict of interest.

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