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ISOLATION SOME PATHOGENIC FUNGI FROM SOME BAGHDAD HOSPITALS AND IDENTIFIED THE MOST FUNGI MOLECULAR FREQUENCY AND USES THE AQUATIC EXTRACT FOR *AGARICUS BISPORUS* FOR TREATMENT

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Abstract

The study collected a hundred samples from November 2018 to February 2019, using sterile transport media swabs from various sources of patients from three hospitals of Baghdad (the medical city, al-yarmuk and al-karama). Ten species were isolated from fungi and the most common genus or species of fungi isolated were *Aspergillus niger* by frequency ratio of 5%, *Candida albicans* by frequency ratio of 3%,*Aspergillus fumigatus* and *Malassezia furfur* by frequency ratio of 2%, *Aspergillus nidulans, Aspergillus terreus, Candida krusei, Candida guilliermondii* and *Candida zeylanoides* by frequency ratio 1%.All fungal isolates were identified depending on the morphological and microscopic examinations, yeast identified by VITEK2 tests, and *Aspergillus niger* identified by molecular (polymerase chain reaction). The obtained results of inhibition effected for aqueous extract with a concentration of 50% on the molds gave the highest inhibitory effect of *Aspergillus flavus*, as the resulting colony diameter was (7) mm and less as a result of *Aspergillus nidulans* as the colony diameter (65) mm, while for the concentration 100% *Aspergillus terreus* gave the highest result, the colony diameter was (1) mm, *Aspergillus fumigatus* was the lowest result, the colony diameter was (38) mm, in concentration 150%, the highest inhibition in *Aspergillus terreus* is (1) mm, and the lowest is inhibition of *Aspergillus nidulans* (50) mm, while the concentration of 200% is the highest inhibition in *Aspergillus niger* where there is no growth and the least inhibition is in *Aspergillus nidulans* was the colony diameter (55) mm, while the obtained results in yeast was as follows, a concentration of 50%, the highest inhibition zone was given in *Malassezia furfur* (20)mm and the lowest in *Candida zeylanoides* (0.33) mm, the concentration of 100% was the highest inhibition in *Candida albicans* and *Malassezia furfur* (20)mm and the least inhibition was in *Candida krusei* (7)mm, while at a concentration of 150% the strongest inhibition was on the *Malassezia furfur* where the inhibition zone diameter was (5) mm and the rest of the species were slightly affected by (0.33)mm, while the concentration of 200% did not give an inhibiting result on yeasts.

Keywords: Pathogenic fungi, fungi molecular frequency, aquatic extract, *Agaricus bisporus*

Introduction

Life-threatening fungemias can result from the introduction of fungal organisms to the bloodstream, with the potential to become a debilitating disease to both community and hospital-based populations. Compared to localized infections (Zaoutis *et al.,* 2010).

The traditional diagnosis of fungal infection depends on identification of pathogens by morphological figures of genus and species. This is sometimes unsuccessful, but because atypical features of some isolates. Molecular Biology identification systems for its pathogenic Aspergillosis as a treat to this problem has been proposed for example, a diagnostic method relies on PCR for detection Aspergillus genus using 18S rDNA. (Makimura *et al.,*1994; Sugita *et al.,* 2004). The aim of this study is identification of fungi from some patients of Baghdad hospitals, treatment some fungi by aqueous extract of *Agaricus bisporus*.

Materials and Methods

Preparation of aqueous extract of *Agaricus bisporus*

The oven dried mushroom are blended, the obtained powder was soaked in D.W. at ratio (1:10)(v/w) and boiled at (60) °C For (30)minutes by water bath, the boiled mushroom powder was then left covered for (30) minutes. Residues were then removed by filtration throw gauze and further centrifuge at (10,000)rpm for (30) minutes, at (4) °C. supernatants were then collected and filtered through whatman (No.1) filter paper. After that, dried extract powders were obtained by using oven at (40)°C till be powder, and stored at (4) °C. Then, the aqueous extract powder would solute in D.W. at ratio (0.5:10)(gm/ml) to prepared the solution with 50% concentration , (1:10)(gm/ml) to prepared the solution with 100% concentration, (1.5:10)(gm/ml) to prepared the solution with 150% concentration, and (2:10)(gm/ml) to prepared the solution with 200% concentration. according to method described by (al-Bahrani,2015).

Identification of fungi

During the incubation period, different fungal colonies were subjected to macroscopic and microscopic to observe their growth, mycelium nature and structure of hyphae. Filamentous fungal growth as mold and yeast that grow on SDA, were sub-cultured on separate SDA culture plates. Pure culture growth of each mold and yeast colony was examined under magnification for their microscopic structures and cross identified by using mycological keys manuals (Koneman *et al*., 1992).

Microscopic and macroscopic examination

In this study, human pathogenic fungi were diagnosed according to (Tille and Forbes, 2014). This identification depends on the following:

1. Colony characteristics (color, consistency and topography).
2. Colony reverses (color, significant pigment).
3. Microscopic morphology (microconidia and macroconidia: their size, shape, arrangement, and hyphal structures).

The yeast identification by VITEK2:

The equipments and instruments used in this technique were listed in the bellow list:

1. VITEK 2 compact
2. Barcode scanner

3. Compact workstation
4. Shared printer
5. Power conditioner
6. UPS (Uninterruptible Power Supply)
7. Test cassettes
8. DensiCHEK™ Plus

Preparation of yeast suspension

A number of colonies of a pure culture were transfer by sterile loop to a plastic tube, and then suspended in 3.0 ml sterile saline (0.50% NaCl) in a clear plastic test tube, then adjusted to (1.80-2.0) McFarland using DensiCHEK™. (Barnett *et al*., 2000).

Inoculation

Identification cards were inoculated with yeast suspensions using a vacuum apparatus. The test suspension tube was placed into the "cassette", while inserting the transfer tube into the suspension tube. The filled cassette was placed into a vacuum chamber station and then the yeast suspension was introduced into micro-channels to fill all the test wells.

The percentage of isolated species from different areas of body.

Source species	Fluid%	Skin%	Blood%	Nail and hair%	Percentage%
A. niger	6	6	1	2	15
A. flavus	2	2	1	Negative	5
A. fumigatus	1	1	Negative	Negative	2
A. nidulns	Negative	1	Negative	Negative	1
A. terreus	Negative	1	Negative	Negative	1
Total mold	9	11	2	2	24
C. albicans	1	Negative	1	1	3
M. furfur	Negative	1	Negative	1	2
C. krusei	Negative	Negative	Negative	1	1
C. guilliermondii	Negative	Negative	Negative	1	1
C. zeylanoides	Negative	Negative	Negative	1	1
Total yeast	1	1	1	5	8
Total infection	10	12	3	7	32

Above table is showed the samples from different sources of patients in some Baghdad hospitals, which have (100) samples collected from different sources of patients such as skin(from different areas of the body), blood, body fluids (urine, cerebrospinal fluid, ascetic fluid, nipple discharge, and fluid pleural), observed some fungi species which cultured on Sabouraud dextrose agar media for (7) days, observed (10) different species of fungi, five species is yeast (*Candida krusi, Candida guilliermondii, Candida zeyalnoides, Candida albicans, Malassezia furfur*) and five species is molds (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulans*). The age range is 50 years, the patient ages between (10-60) years old. Each variety (skin, nail and hair, blood, fluids of bodies) contains 25 samples. *A. niger* was the most frequently of pathogenic fungi which made the infection of patients While Diba was disagree because he had different results, he concluded *A. fumigatus* was more frequently than other species (Diba *et al.,* 2007). According to the results, the skin is the most affected area. The incidence of infection in the area of body fluids is similar to the incidence of injury to the skin. The blood is the lowest place where infectious fungi have occurred. From the results shown in the table, it was found that mold infection is more than yeast infection.

Fungi Diagnosis

- **Molds:** The molds diagnosed by macroscopic diagnosis by observing the shape and color of the colonyon the culture medium and microscopic diagnosis by observing the shape, size and distribution of the conidia and the type of conidia, if it consisted of septate or not.
- **Yeast:** The yeasts identification by the external morphology of colonies and by (64) test of VITEK2.

Malassezia furfur:

The colony morphology is showed single larger colony with average (2-3) mm diameter, comprised white to cream colored. Circular with irregular margins, friable, soft, smooth and no specific smell. (Manna *et al*.,2015).

Identification by VITEK2.

Gene detection:

The result of molecular detection of *Aspergilus niger* were illustrated in Figure (1).

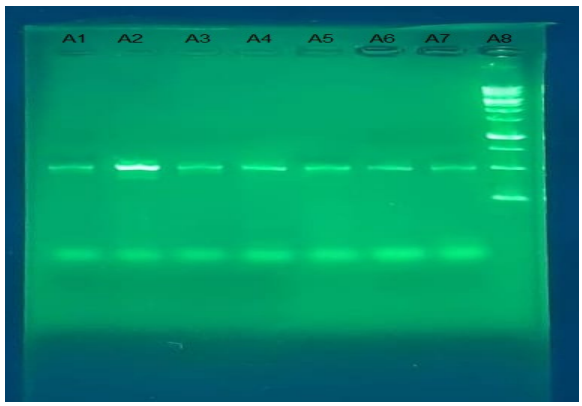

Fig. 1 : Gel electrophoresis for DNA *A. niger* (A1-A7)= DNA of *A. niger,* A8=ladder

All seven tested isolate were positive for ASAP (ASAP1-ASAP2) gene

ASAP was located in all seven isolates of *Aspergillus niger* at 521 bp

All seven tested isolate were positive for Nig which composed of (ASPU and Nilr) gene.

Nig was located in all seven isolates of *Aspergillus niger* at 521 bp

This specific gene used by Sugita and confirmed this specific gene was successful in amplifying only the target species (Sugita *et al.,* 2004).

The aqueous extract of *A. bisporus* against the fungi:

The aqueous extract had an effect on most isolated fungi as it had a inhibitory effect. Since the extract was used in different concentrations on fungal species, it was found that some concentrations resulted in good inhibition and other concentrations that did not give the same effect on the fungal species, while some species responded more than the other species for the same concentration. It should be noted that the concentrations of (50%) and (100%) gave strong and clear results on yeasts, while concentrations of (150%) and (200%) did not produce acceptable results in the inhibition of yeasts used in this study. unlike molds, their response varied to different concentrations of aqueous extract of *A. bisporus.* These results are consistent with Waithaka where he was proved in 2017 that the extract of the mushroom gives inhibition results on microorganisms. (Waithaka *et al.,* 2017).

The aqueous extract of *A. bisporus* against molds:

Table 1 : Show the effect of aqueous extract of *Agaricus bisporus* with concentrations (50-100-150-200)% against isolated species.

Mold / Concentrations	Aspergillus niger	Aspergillus nidulans	Aspergillus funigatus	Aspergillus terrrus	Aspergillus flavus
Control	22.33 c ± 2.60	22.33 d ± 2.60	22.33 c ± 2.60	22.33 c ± 2.60	22.33 c ± 2.60
50 % µg/ml	37.00 a ± 0.76	65.00 a ± 0.29	25.00 c ± 0.17	39.00 b ± 1.15	7.00 e ± 0.58
100 % µg/ml	30.00 b ± 0.66	23.00 d ± 1.15	38.00 a ± 0.29	1.00 d ± 0.06	34.00 a ± 1.15
150 % µg/ml	32.00 b ± 0.58	50.00 c ± 0.58	33.00 b ± 0.58	47.00 a ± 0.58	17.00 d ± 0.53
200 % µg/ml	0.00 d ± 0.00	55.00 b ± 1.00	33.00 b ± 0.43	50.00 a ± 1.15	27.00 b ± 0.75
LSD P ≤ 0.05	4.02	4.35	3.84	4.41	4.29

Above table showed the aqueous extract of *Agaricus bisporus* and control against five species of molds. The aqueous extract of (50%) was used, *A. flavus* was more responsive than other species. While, *A. nidulans* were less responsive than other species, and the response of *A. Niger* and *A. terreus* was an approach to each other. The concentration of (100%) gave the highest inhibitory result in *A. terreus* while in *A. fumigatus* it was less inhibition and the inhibitory result in *A. flavus* was very close to that in *A. fumigatus.*

The obvious difference can be seen by the effect of this concentration on *A. terreus* from other species. In the (150%) concentration, the highest inhibitory result was found in *A. flavus* compared to other species, While they were less discouraged in *A. nidulans*, the result was very close to the result of this emphasis on *A. terreus.* It can be seen that the inhibitory results of this concentration on the colonies of the species *A. niger* and *A. fumigatus* gave convergent inhibition results.

Finally, in talking about the effect of concentrations on the types of molds used in this study, the concentration of(200%) gave more inhibitory result in *A. niger* compared with other species, and less inhibition in *A. nidulans* then followed in *A. terreus.* After observing the table it could be seen that the highest inhibition is in the aqueous extract with a concentration of (100%) on *A. terreus* Ammar in 2019 studed the effect of aqueous extract of *A. bisporus* on some microorganisms and demonstrated that extract had inhibitor effect on some microorganisms (Ammar, 2019).

The aqueous extract of *A. bisporus* effect against *A. niger:*

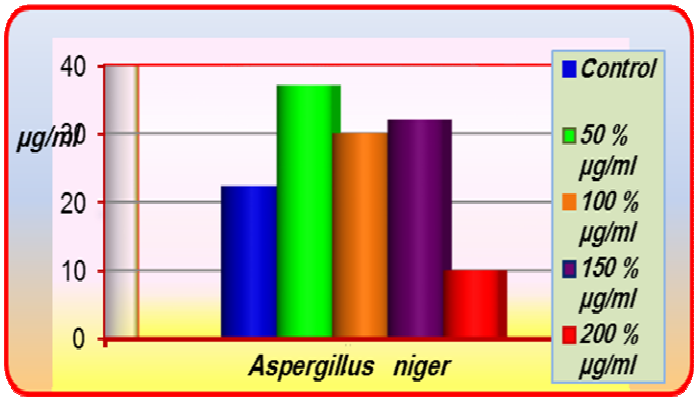

Fig. 2 : The effect of aqueous extract of *Agaricus bisporus* with concentrations (50-100-150-200)%against *Aspergillus niger*

The Figure is showed the effect of aqueous extract of *A. bisporus* on *A. niger,* The concentration of (200%) was most influential on this fungal species with the highest inhibition Compared with other concentrations. While, the concentration is (150%), the result is the closest to the concentration of (50%), while the concentration of (50%) gave less inhibitory result compared with other concentrations used. This result agree with Han who

demonstrated that the mushroom had inhibitory effect on *A.niger.*(Han *et al.,2008).*

The aqueous extract of *A. bisporus* effect against *A. terreus:*

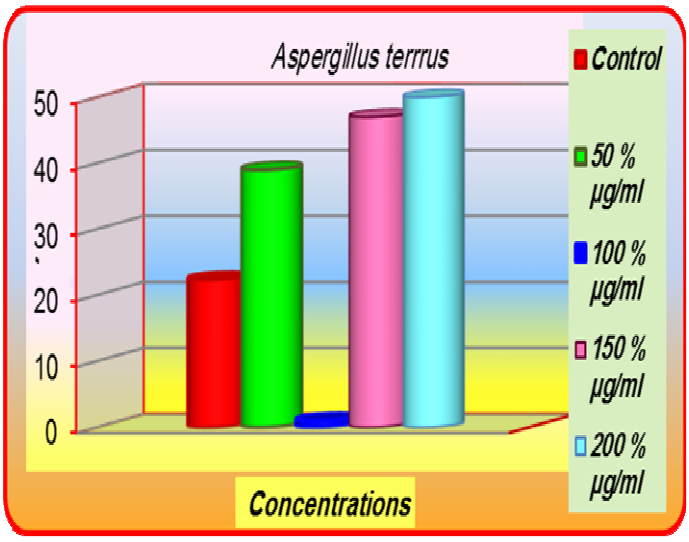

Fig. 3 : The effect of aqueous extract of *A. bisporus* with different concentrations against *A. terreus*.

In this Figure, the difference in the response of the *A. terreus* to the effect of aqueous extract of *A. bisporus* was illustrated. The concentration of (100%) had a greater inhibitory effect than other concentrations, while the concentration of (200%) had the lowest inhibitory effect on this fungal species, this difference was calculated by measuring the diameter of the colony. It was observed that the effect of concentrations of (150%) and (200%) had almost similar results.

The aqueous extract of *A. bisporus* against *A. flavus:*

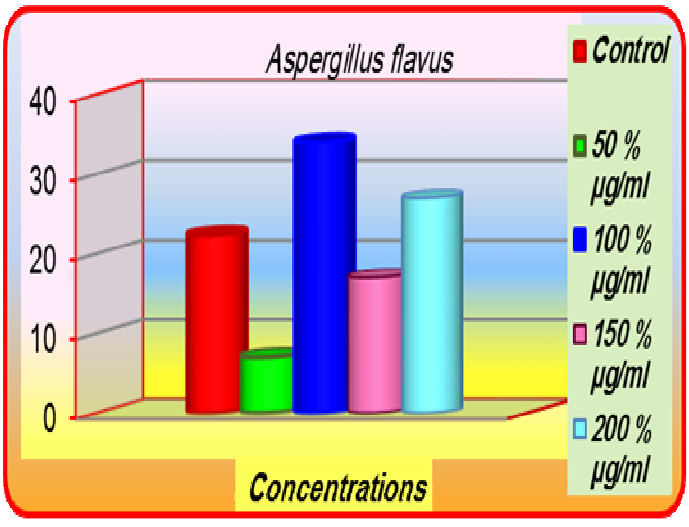

Fig. 4 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Aspergillus flavus.*

In this Figure, the results of the aqueous extract of *A. bisporus* were illustrated on *A. flavus* where the concentration of (50%) gave the highest inhibitory rate compared to the other concentrations. While the concentration of (100%) gave less inhibitory result on this fungal species.

Kumar demonstrated that the inhibitory effect of *A. bisporus* on *A. flavus. (*Kumar and Yadav, 2014).

The aqueous extract of *A. bisporus* effect against *A. fumigatus:*

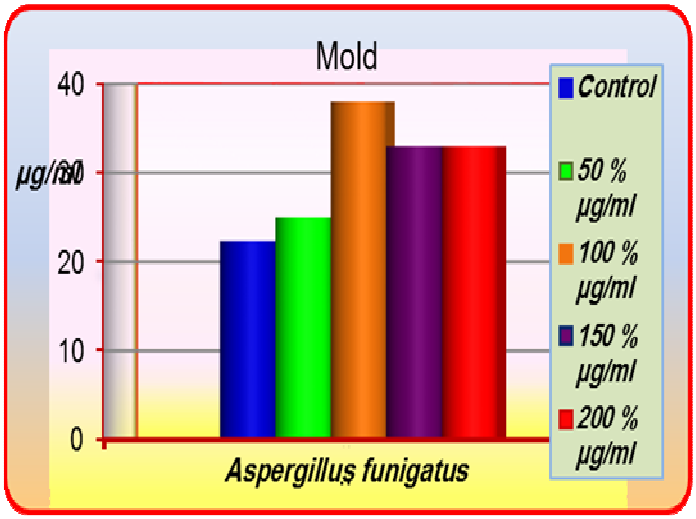

Fig. 5 : The effect of aqueous extract of *A. bisporus* with different concentrations against

Aspergillus fumigatus

This Figure shows the effect of aqueous extract of *A. bisporus* on *A. fumigatus.* It was found that the concentration of (50%) gave the highest inhibitory rate comparable to another concentrations by the small diameter of the colony resulting, While concentrations of (150%) and (200%) gave very similar results to each other. The colony growing in concentration conditions (100%) gave the lowest inhibition rate.

The aqueous extract of *A. bisporus* against *A. nidulans:*

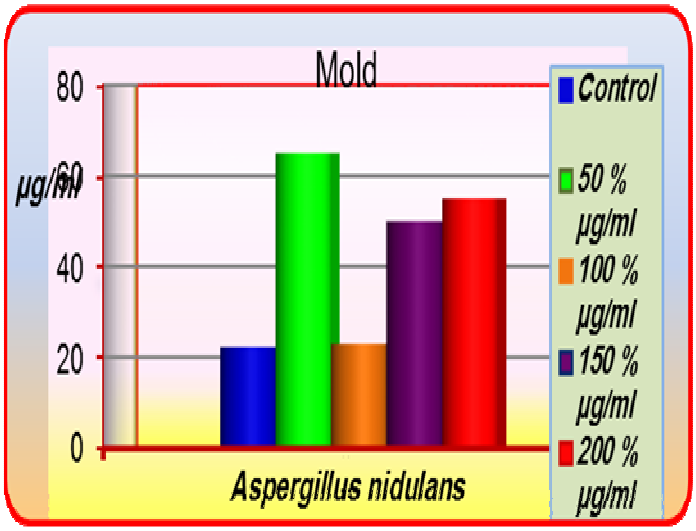

Fig. 6 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Aspergillus nidulans*.

In this Figure, the difference in response to the different concentrations of aqueous extract of mushrooms is illustrated. The concentration of (100%) gave the highest response result as the colony diameter was lower than others of the colonies growth in the other concentrations. It can be seen that the concentration of (50%) gave less result as the colony growing in the medium containing this concentration was larger than the other colonies growth in other concentrations. While, the results of concentrations of (150%) and (200%) were closely related to the colonies of this fungal species, the results can be considered to be close to the concentration of (50%).

The aqueous extract of *A. bisporus* effect against yeast:

Table 2 : The effect of the aqueous extract of *Agaricus bisporus* against yeasts

Yeast / Concentrations	Candida krusei	Candida guillermondi	Candida zeylanoides	Candida albicans	Malassezia furfur
Control	0.50 b ± 0.06	0.50 b ± 0.06	0.50 b ± 0.06	0.50 c ± 0.06	0.50 c ± 0.06
50 % µg/ml	9.00 a ± 1.15	15.00 a ± 0.58	0.33 b ± 0.03	18.00 b ± 0.58	20.00 a ± 1.15
100 % µg/ml	7.00 a ± 1.15	16.00 a ± 0.58	14.00 a ± 0.58	20.00 a ± 0.50	20.00 a ± 1.15
150 % µg/ml	0.27 b ± 0.09	0.33 b ± 0.03	0.33 b ± 0.02	0.30 c ± 0.06	5.00 b ± 1.15
200 % µg/ml	0.13 b ± 0.03	0.13 b ± 0.03	0.13 b ± 0.03	0.13 c ± 0.03	0.13 c ± 0.03
LSD P ≤ 0.05	2.31	1.16	0.83	1.08	2.82

This table is showed the results of aqueous extract of *A. bisporus* and control against five species of yeasts which used in this study. The obvious difference was observed with (50%) and (100%) concentrations giving the highest inhibitory results for all species used for the study, this mean that these two concentrations are the most effective in yeasts.

This table () observed that the concentration of (50%) gave the highest inhibitory results in *Malassezia furfur*, While other species such as *Candida albicans, Candida krusei* and *Candida guilliermondii* gave clear inhibition results except *Candida zeylanoides* did not result in a clear inhibition as shown in the Figure. The(100%) concentration of aqueous extract of *Agaricus bisporus* gave a clear inhibition on *Malassezia furfur* and *Candida albicans*, which was considered the strongest inhibition result for the other species. Inhibition results in these two types are close together, While in *Krusei* gave less inhibitory result. The results of *C. guilliermondii* and *C. zeylanoides* were convergent. since reading the results of (150%) concentration of aqueous extract of *Agaricus bisporus* on the species of yeast used, it was noted that the concentration of (150%) did not give clear inhibition results except in the species *Malassezia furfur.*

The concentration of (200%) of the aqueous extract of *Agaricus bisporus* did not give any clear inhibition results also on all types of yeasts on which the aqueous extract of *Agaricus bisporus* was applied.

The effect of the aqueous extract of *A. bisporus* against *Candida albicans:*

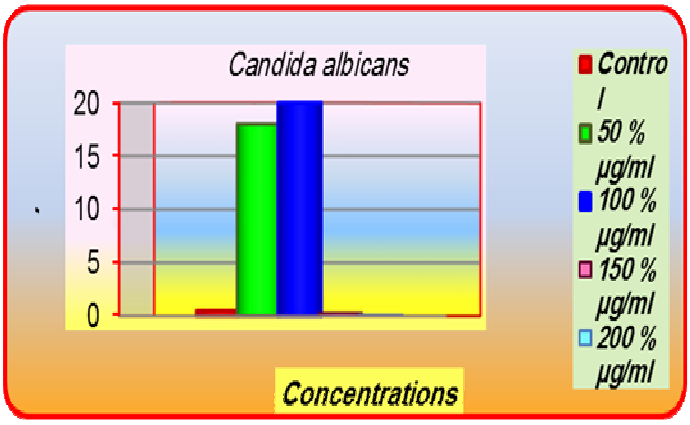

Fig. 7 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Candida albicans.*

After reading the results in this chart it was observed that the highest inhibition of this yeast was obtained at the concentration of (100%) of the aqueous extract of *A. bisporus* as this concentration gave the highest result.

Followed by strongly inhibitory activity is (50%) concentration, As it has a close inhibition to the (100%) concentration inhibition. The remaining concentrations of (150%) and (200%) did not produce clear inhibition results in this species.

This result was agree with Paccola results who demonstrated that the mushroom had inhibitory effect on *Candida albicans.* (Paccola *et al.,* 2001). While disagree with Alves which demonstrated this species had no inhibitory effect by mushroom. (Alves *et al.,* 2013).

The effect of the aqueous extract of *A. bisporus* against *Candida krusei:*

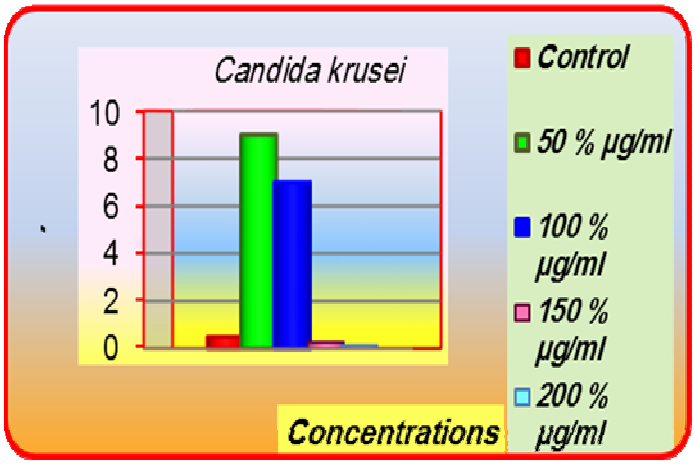

Fig. 8 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Candida krusei.*

During the reading of the diagram it was observed that the highest inhibitory result was obtained in the (50%) concentration of the aqueous extract of *A. bisporus* as shown in the previous figure followed by the effective (100%) concentration.

While the remaining concentrations were (150%) and (200%) of aqueous extract of *A. bisporus*, their inhibition results were almost non-existent, this means that the (50%) and (100%) concentrations of aqueous extract of *A. bisporus* had a clear effect on the cell.

This result agree with Alves and Rosa who demonstrated the inhibitory effect of mushroom on *C. krusei.* (Alves *et al.,* 2013; Rosa *et al.,* 2003).

The effect of the aqueous extract of *A. bisporus* against *Candida guilliermondii:*

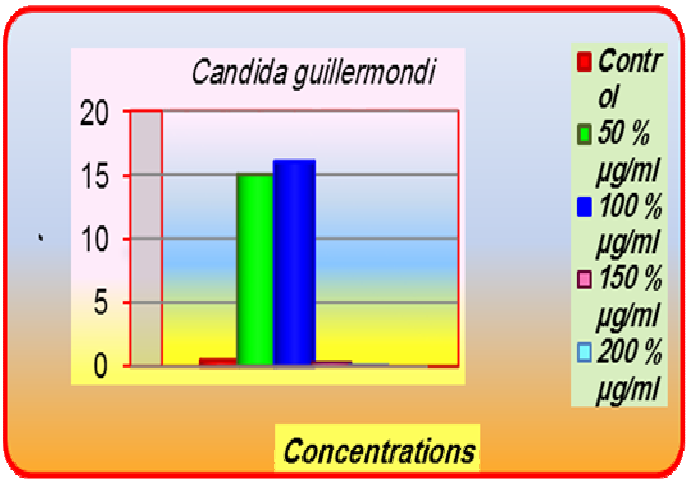

Fig. 9 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Candida guilliermondii*

This Figure showed also in this yeast type *C. guilliermondii* it was observed that the concentrations (50%) and (100%) are remarkably effective without the other concentrations (150%) and (200%) of *A. bisporus* aqueous extract.

The highest inhibitory result was in aqueous extract with (100%) concentration followed by (50%) concentration.

The aqueous extract of *A. bisporus* with concentrations of (150%) and (200%) had no effect on this species.

The effect of the aqueous extract of *A. bisporus* against *Candida zeylanoides:*

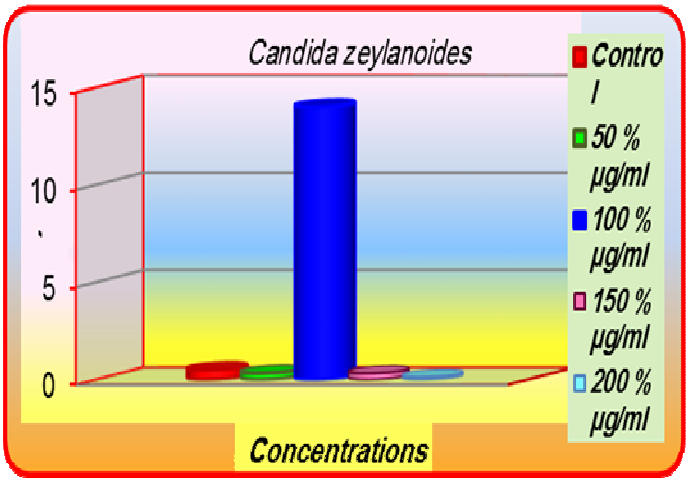

Fig. 10 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Candida zeylanoides*

since reading the previous Figure it was observed that this yeast species did not respond to the inhibition of any concentration used from the aqueous extract of the mushrooms except the concentration of (100%) has been given a discouraging result, this result was observed by observing the inhibitory region obtained after the application of this extract on it. It should be noted that the concentrations of (50%), (150%) and (200%) did not produce a reliable inhibitory result.

The aqueous extract of *A. bisporus* effect against *Malassezia furfur:*

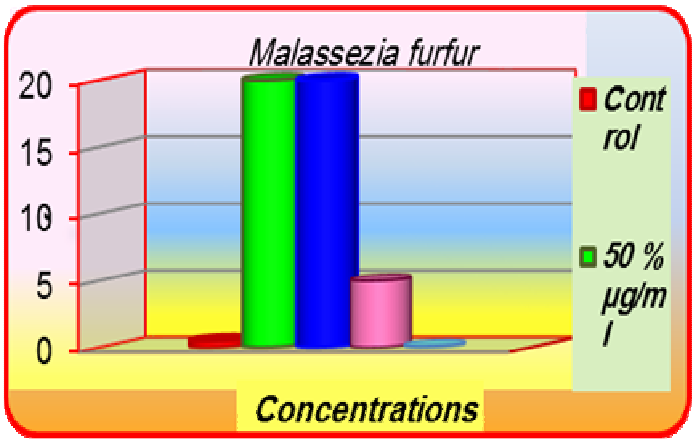

Fig. 11 : The effect of aqueous extract of *A. bisporus* with different concentrations against *Malassezia furfur.*

This Figure shows the effect of aqueous extract of *A. bisporus* on this yeast species (*M. furfur).*As shown in the previous Figure, the aqueous extract of (100%) and (50%) concentration gave very close results from each other. It is noticeable that in this yeast type also the concentration of (50%) and (100%) is the most inhibition of other concentrations. However, it was noted that the concentration of (150%) gave a discouraging result compared with its results in the remaining species used in this study, But still a weak result compared to (50%) and (100%) concentration of aqueous extract of *A. bisporus.*

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