



Evaluation of the efficiency of *Pleurotus Ostreatus* in the percentage of inhibition of fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Penicillium sp.*

Hussein Sami Najji Kermasha ^{1*}

¹ Faculty of Education for Girls, University of Kufa, Al-Najaf Governorate, IRAQ

*Corresponding author: Husseinsaeed1976@gmail.com

Abstract

The study evaluated the efficacy of *Pleurotus Ostreatus* in the percentage of inhibition of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Penicillium sp.* The fungal was growth in The laboratory and in enbated at atemperature (25±2) in the microbiology laboratory in the Department of Life Sciences / College of Education for Girls, The experiments were carried out on 2-9-2019 and ended on 2-2- 2020. The results showed a significant difference in the exudates of the *P.ostreatus* for ages (7,21,28) days, Streatus (28) days superior to the fungal inhibition rate *A.niger* at all ages was 97% compared to control of 0%, while the lowest percentage of inhibition of fungus *A. niger* by treatment of exudates mushroom *P. ostreatus* at the age of (7) days was 88%.The results of the treatment of exudates *P. ostreatus* mushroom at the age of (21, 28) days was significantly higher in the fungus *A. flavus*, where it was 94% compared to the control treatment of 0%, while the lowest inhibition of the fungus *A. flavus* with the treatment of exudate. *P. ostreatus* at 7 days 93%. The treatment of exudate fungus *P. ostreatus* at 28 days significantly differentiated in *A. terreus* fungus on all ages, reaching 96% compared to control treatment where it was 0%, while the lowest percentage of inhibition of diameter *A. terreus* treatment of exudate fungus *P.ostreatus* at 7 days, where it was 89%.The results of treatment of fungus *P. ostreatus* 7 days was significantly higher in the fungal suppression rate, *penicillium sp.* at all ages was 90% compared to control of 0%, while the lowest percentage of inhibition of fungi *penicillium sp.* in the treatment of exudate fungus *P. ostreatus* at a 21-day age where it was 88%.

Keywords: exudates *Pleurotus Ostreatus*, ages (7, 21, 28) days, different fungus

Kermasha HSN (2020) Evaluation of the efficiency of *Pleurotus Ostreatus* in the percentage of inhibition of fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Penicillium sp.* Eurasia J Biosci 14: 4783-4787.

© 2020 Kermasha

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

INTRODUCTION

The oyster mushroom *Pleurotus ostreatus* is an important basidial fungus whose body contains a high percentage of substances, such as proteins and carbohydrates, and does not contain fats, and if they contain it, it is in very few proportions, as well as containing vitamins, salts and other nutrients (Manolea et al, 2006).

As the sources indicated its importance in many fields such as economic and medical in different colors, types and places of cultivation, the range of its efficiency varies according to the type of supports that are added to the agricultural media such as molasses, yeast, wheat bran and some types of extracts such as licorice, which helps to focus some important medical chemicals in the body of the mushroom like oysters. Cystatin, beta-clocane and kalic (Saad et al, 2013). Also, many studies and research have confirmed the importance of oyster mushrooms in inhibiting many pathogenic fungi, viruses, and bacteria and have shown its role in analyzing

mycotoxins such as toxins produced by the fungus *Aspergillus Fumigatus*, as well as its role in analyzing blood clots and reducing cholesterol in animal bodies (Ibrahim et al,2005). The fungus *Aspergillus niger* is a common fungus of the genus *Aspergillus*. It is colonies of black to brown color. Conidiophore is long with smooth, transparent walls and is dark at its summit. The onset of growth is divided into a few columns as they age (perrone et al, 2007). Mushrooms are commonly found in warm environments in the field as well as stored foodstuffs and grow on vegetables and fruits, causing what is known as black mold and enter in many industries where it produces some enzymes and organic acids, and its spores have a high resistance against ultraviolet radiation as well as sunlight Abara et al, 2004).

Received: July 2019

Accepted: March 2020

Printed: October 2020

As for the fungus *Aspergillus flavus*, it is one of the common fungi in nature that was described for the first time by the world (Link) in 1908. Ideal for its growth, and its growth can be observed with a temperature ranging between (12-48) A.D. (Ahmed, Wefaq, 2015) for fungi being susceptible to causing various diseases as it causes human, animal and plant injury as a result of its ability to produce fungal toxins such as cyclopiazonic acid and aflatoxin (Aflatoxin) and Aflatoxin, the last substance of pain Rtunh and mutagens also (wang et al,2016). In man, for example, respiratory diseases, including several hypersensitivity and sinus infections and also causes skin infections disease (Hedayati et al,2007). The fungus *Aspergillus terreus* is one of the widespread fungi as it is isolated from the soil and the remains of plants and the surrounding air also has the ability to grow in harsh environmental conditions where it can grow at body temperature or higher than it and also grow in a humid atmosphere with humidity up to 60 and this type is considered One of the fungi is one of the least fungi that causes opportunistic diseases (Ahmed, Wefaq,2015) and it affects people who suffer from weak immune system in particular, it affects people with cancer who are receiving chemotherapy or patients with AIDS (Tomc and churc,1918)) And also be responsible for the injury respiratory disease infections, also causes some superficial skin diseases such as cracking nails (Onycho mycosis) (Lass-flori et al, 2005) (SHimada et al, 2002).

Fungi *Penicillium* sp is a widespread fungus, found wherever decomposing organic matter is found and its spores are ubiquitous and abundant in soil (Al-Suhaili et al, 1980, Khudair, 1987). *Penicillium* is one of the fungi that have the ability to withstand different ranges of temperatures and this justifies its appearance in most seasons of the year (Hamad, 1998) and in a study by (Abdel Hafez,1981) on salinity tolerant fungi in desert soils it was found that the most tolerant species, *Aspergillus* and *Penicillium* sp and Hamad (1998) found that *Penicillium* sp. He demonstrated his dominance in

MATERIALS AND METHOD

This study included laboratory experiments A pure isolation of mycelium of oyster mushroom (*Pleurotus ostreatus*) was obtained from A. Dr. Majid Meteeb Diwan of the Fungi Laboratory in the Department of Plant Protection - College of Agriculture, University of Kufa and identified according to the classification key (Singer, 1986), while the fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium* sp) were obtained from the Laboratory of Microbiology where It was isolated from the soil by means of dilution and was classified with the help of. Dr. Hussein Sami Najji The isolated fungi were diagnosed based on the characteristics mentioned in the classification keys (Geiser, 2009) and Kirk et al, 2008).

Agricultural media used in conducting experiments

Potato Dextrose Agar in a potato, dextrose and ready-made agar (P. D. A)

Prepare by dissolving 39 g per liter of distilled water according to the instructions of the producing company, then add the antibiotic (Amoxicillin) in the amount of 250 mg / liter. Distribute the medium in 250-ml glass jewels, fill their nozzles with tampons and sterilize the dressing device at 121 C°. and press 15 pounds / 2ng for 20 minutes. After sterilization is complete, the jugs are left to cool to before solidification, then pour the medium into dishes and store in the refrigerator at 4 C° until Use. This medium was used to develop, isolate, and classify isolated and contrasting fungi among them.

Potato Dextrose Broth medium (P. D. B)

The center attended in the same way that the center (P. D. A) attended, but without adding the nests Distribute the prepared medium into 10 glass bottles, each of 250 ml capacity of 150 ml for each bottle, and sterilize the previous method and use this medium to prepare the oyster mushroom filter where the bottles were vaccinated separately, by placing 3 diameter tablets (0.5) cm each Avial of *P.ostreatus* oyster mushroom grown on (7) day on (P.D.A) nutritional medium .The bottles are incubated in the incubator at (30 ± 2 C°) for a period of (7, 21, 28) days (Dewan, 1989). After completing the bosom period, it was filtered by Wtman.NO.1 filter paper to get rid of impurities and to obtain a clear solution, then pass using a 0.22 millimeter Millipore filter to get rid of the fungus boards and get a pure filtrate .After that, the filtrate was stored according to the ages mentioned above in the refrigerator until use. After that, the leachate was taken and distributed in the pits, adding 0.5 ml fungal leachate per hole according to the ages. 3 replications were made for each age and for each fungus.

Method of inoculation and inhibition of fungal isolates and calculation of percentage of inhibition

The Well diffusion method (Al-rammahi, 2015) was adopted by making three pits(0.5) mm in diameter using a sterile cork punch using equal dimensions from the center in the culture medium (P.D.A) followed by adding (0.5) ml of the *P. streatus* filtrate. *P. ostreatus* ages (7, 21, 28) days using a medical syringe and three replicates with three replicates of dishes containing sterile culture medium without the addition of the oyster mushroom *P. ostreatus* as a comparison (Control) by adding distilled water only.

Then all the dishes containing the fungi were vaccinated by placing a disk with a diameter of (5) mm in the center of the veterinary dish taken from the edge of the colony of the fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium* sp at the age of

(7) days, and incubated the dishes at a temperature of $(25 \pm 2 \text{ C}^\circ)$ For a period of (72) hours, it was followed by measuring the Inhibition Zones using a measuring ruler, then taking the measurement rate and calculating the percentage of inhibition according to the following equation (Al-khafagy, 2016).

$$\text{Inhibition} = \frac{C - T}{C} \times 100 \%$$

Where C: rate of mushroom growth diameter (comparison factor)
T: rate of growth of fungus in dishes containing *P.ostreatus* (treatment)

Isolation purification and diagnosis of fungi

Random samples were taken from different places of agricultural soils in the College of Education for Girls and then took (10)g of soil and added to a beaker containing (90) ml sterile distilled water and mixed by shaking, then took (1) ml from it and added to a test tube containing (9) ml sterile distilled water to get Focus on (10^{-2}) Then I made a series of scares until the fourth and fifth dilutions were reached $(10^{-4}$ and $10^{-5})$.

Then we took (1)ml of each of the previous two tubes and put them in a sterile plastic Petri dish with a diameter of (9) cm, adding (20) ml of the food medium(P.D.A.) Sterilizer added to the antibiotic (Amoxicillin) at 250 mg / l before its solidification and at a temperature of 45 C° .

Repeat the process three times for each dilution and move the plates a molar movement, and leave the plates to harden and then incubated incubator inverted form at a temperature of $(2 \pm 25\text{C}^\circ)$ for a period of (7) days. Then the fungi were isolated and then individually purified on Petri dishes containing (20) ml of(P.D.A.) food medium.

Statistical analysis

The data of the country growth rate, the inhibition rates of isolated fungi and pathogenicity tests were analyzed using Completely Randomized Design according to practical experiments with two factors. The averages were reviewed according to the method of the least significant mean difference (L.S.D) .And the probability level is 0.05 (Narrator and Khalaf Allah, 2000).

RESULTS AND DISCUSSION

Effect of *P.ostreatus* filtrate on a lifetime of (7) on the percentage of inhibition of fungi *A.niger*, *A.flavus*, *A.terreus*, *Penicillium* sp

From **Table 1** the effect of a 7-day-old oyster filtrate *P.ostreatus* on the percentage of inhibition of the studied fungi, it is clear that the percentage of inhibition of the fungus *A.flavus* is the highest inhibition rate as it reached 93% compared to the control which reached 0% and the lowest inhibition rate was for the *A.niger* reached 88%, while fungus *Penicillium* sp.)*A.terreus* were (90%, 89%) respectively.

Table 1. Effect of *P.ostreatus* on the age of (7) on a percentage of inhibition of fungi *A.niger*, *A.flavus*, *A.terreus*, *Penicillium* sp

Fungi	Fungus Inhibition%
<i>Aspergillus niger</i>	88%
Control	0%
<i>Aspergillus flavus</i>	93%
Control	0%
<i>Aspergillus terreus</i>	89%
Control	0%
<i>Penicillium</i> sp	90%
Control	0%
L.S.D. 0.05	2.9

Table 2. Effect of *P.ostreatus* at the age of (21) on a percentage of inhibition of fungi *A.niger*, *A.flavus*, *A.terreus*, *Penicillium* sp

Fungi	Fungus Inhibition%
<i>Aspergillus niger</i>	93%
Control	0%
<i>Aspergillus flavus</i>	94%
Control	0%
<i>Aspergillus terreus</i>	92%
Control	0%
<i>Penicillium</i> sp.	88%
Control	0%
L.S.D. 0.05	2.2

These results recorded in the experiment are consistent with what he found (Stamets,1993) (Koch and Buchalo, 2002) that confirm that the enzymatic filtrate of the shellfish contains active compounds and enzymatic extracts that have an active role that can influence the functional effect on the fungal yarn of the fungi .This is what gives him medical importance. Also, the results can be explained in light of what he said (Wood et al, 2000; Stamets, 2001; Chase et al, 2003) in that the oyster mushroom is distinguished by its presence of antimicrobial and antifungal agents.

Effect of *P.ostreatus* filtrate leachate at age (21) on the fungi inhibition ratio *A. niger*, *A. flavus*, *A.terreus*, *Penicillium* sp.

While **Table 2** was the effect of *P.ostreatus* filtrate leachate at the age of (21) days on the percentage of inhibition of the aforementioned fungi, the percentage of inhibition of the fungus *A.flavus* was the highest inhibition rate where it was 94% compared to control where it reached 0% and the lowest inhibition rate was for the fungus *Penicillium* sp This was 88%, and the fungicide inhibition rates *A. niger*, *A.terreus* were (93%, 92%) respectively. These effects may be explained on the basis that some of the filters may contain one or more compounds of carbohydrates, peroxidase and pectase enzymes, fatty acids, amino acids, or toxic substances (Al-Amiri, 2011).

Also, the effect of isolated fungi filtrates on Qatari growth may explain the filtration of toxins that adversely affect fungi growth (Al-Rikabi, 2008). Fungi are characterized by their ability to produce secondary metabolic compounds when they grow in a suitable environment for them and these compounds are bioactive according (Saad, 1991). The reason may be

Table 3. The effect of *P.ostreatus* filtrate leachate at 28 years of age on the fungi inhibition ratio *A.niger*, *A.flavus*, *A.terreus* and *Penicillium* sp

Fungi	Fungus Inhibition%
<i>Aspergillus niger</i>	97%
Control	0%
<i>Aspergillus flavus</i>	94%
Control	0%
<i>Aspergillus terreus</i>	96%
Control	0%
<i>Penicillium</i> sp.	89%
Control	0%
L.S.D. 0.05	3.1

attributed to the ability of fungi to secrete some catalysts or increase the readiness of some nutrients (Al-Jafari, 2006).

Influence of Oyster filtrate *P.ostreatus* at the age of 28 days on the inhibition of fungi *A. niger*, *A. flavus*, *A.terreus*, *Penicillium* sp

As for **Table 3**, the effect of (28) day-old oyster filtrate of *P.ostreatus* on the percentage of inhibition of studied fungi, it was revealed that the highest inhibition rate was for *A.niger* where it reached 97% compared to the control treatment of 0% and the lowest inhibition was *Penicillium* sp where it was 89% and the percentage of inhibition of fungi *A.terreus*, *A.flavus* was (96%, 94%) respectively. This is confirmed by (Al-Haidari, 2014) when studying the interference between the filtrate filtrate factor isolated and the time factor and its effect on the Qatari growth, we note that the filtrate fungus *P. nigricans* and *P.camembertii* have worked to reduce the Qatari growth of the *F.graminearum* fungus after 72 hours as it reached 3.63 and 3.70 cm, respectively.

REFERENCES

- Abara, M.L; Accensi, F; Cano,J; Cabanes,F.J. 2004. " Taxonomy and significance of black aspergilli. Antonie Van Leeuwenhoek. 86:33-49.
- Abdel Hafez, S.I.I. 1981. Osmophilic fungi of desert soils in Saudi Arabia. Mycopathologia 80: 9-14.
- Ahmed, Wefaq.2015. Isolation and diagnosis of some external ear molds and ability test MA, Department of Life Sciences, College of Education for Girls, University of Kufa, 2015.The fungus *Aspergillus* secrete some enzyme virulence factors "letter
- Al-Haidari, Firas Hadi and Sen.2014. The effect of filtrates of some fungi isolated from rice residues on germination of wheat seeds and growth of pathogenic fungi *Fusarium graminearum*, *Rhizoctonia solani* and resistance fungicide *Trichoderma harzianum* in vitro. Master Thesis, College of Agriculture, University of Kufa.
- Al-Jaafari, Wissam Adnan Radhi.2006. isolation and diagnosis of fungi associated with rice seeds treated with fungicide *Trichoderma harzianum* Rafi under storage at different moisture levels and their effect on germination and seedling growth. Master Thesis. College of Agriculture. University of Kufa.
- Al-khafagy A.H.,.2016. "Synthesis, Characterization and biological study of some new metal-azo chelate complexes " J. of Chem. and pharm. Res., 8(8), pp. 296-302.
- Al-rammahi A.S., Al-khafagy A.H. and Al-rammahi F.,.2015. Synthesis and Characterization of oxazepin and imidazolin derivatives from 2-amino -5- mercapto-1,3,4-thiadiazol and studing of their biological activity " world. J. of pharm. Res.,4(2). pp. 1668 -1680.
- Al-Rikabi, Firas Ali Ahmad.2008. The effect of vegetative growth extracts of some bushes on pathogenic fungi on bio-resistance fungi. Master Thesis, College of Agriculture, University of Kufa.
- Al-Suhaili, Ibrahim Aziz Khaled, Qaisar Najeeb Saleh and Abdul-Taif Salem Ismail.1980. Fungi. Dar Al-Kutub for Printing and Publishing - University of Mosul Page: 320.
- Chase, Ch. Garner, M. Graves, D. Oliff, H.S. Schulman, R.N. and D. Webb. 2003. Major Review of health Benefits of Medicinal Mushrooms. Mushroom Medicinal. www.herbalgram.org
- Dewan, M.M. 1989. Identity and frequency occurrence of fungi in root of wheat and rye grass and their effect on take_all and host groth.Ph.D. thesis univ. Western Australia., 210.
- Geiser, D. 2009. "Sexual structures in *Aspergillus*: morphology, importance and genomics " Medical Mycology: official publication of the International Society for Human and Animal Mycology. 47 Suppl 1 (s1): S21-S26.
- Hamad, Nida Shehab.1980. Desert Society of microscopic fungi in the soil of the regions of Iraq. PhD thesis - College of Science - University of Babylon.
- Ibrahim, Diaan Khalil, Sonbol Jassem Hammoudi and Muhammad Ahmed Shwail al-Mashhadani.2005. The effect of adding oyster mushrooms to the diet of male broiler chicks on some traits Department of Animal Resources - College of Physiological and productive. Agriculture - University of Baghdad.. research Mistle from a master's thesis.. Iraqi Journal of Agricultural Sciences 19 (3): pp. 20-29.

- Khudair, Abdul Hamid Khaled.1987. General plant diseases. Dar Al Kutub Printing Press And Publishing - University of Mosul.
- Kirk, P.M.;Cannon, P.F.;Minter,D.W.;Stalpers, J.A. 2008.Dictionary of the fungi (10th ed).Wallingford,U.K.P:505-978.
- Koch. J.S.Witt and U.Lindeguist.2002. The influence of selected higher basidiomycetes on the binding of Lipopolysaccharide to CD +14 cells and on release of cytokines. international Journal of Medicinal mushrooms. 3:94 -101.
- Lass-Flori, Cornelia, Griff K., Mayr A., Petzer A., Gastl G., Bonatti H., Freund M., Kropshofer G., Dierich M., Nachbaur D.,.2005. "Epidemiology and outcome of infections due to *Aspergillus terreus*: 10-year single center experience " British J. of Haematology. 131 (2) pp.20-207.
- Manolea, G. Popescu, M. Nedelcut, C. and Alboteanu, L. 2006. The numerical simulation of the culture medium for the *Pleurotus* genus mushrooms, Ann. Uni. Craiova, Elect. Engin. Seri., 30: 318-325.
- Perron, G. Susca, A. Cozzi, G. Ehrlich, K. Varga, J. et.al.2007. Biodiversity of *Aspergillus* spp. in some important agricultural products Stud. Mycol.:53-66.
- Saad, Magdy Moheb El-Din Mohamed.1991. Mycotoxins, Agricultural Problem, Surface Surface. Egyptian Book Authority, Cairo
- Saad, Samah, Nidal, Soufan, and Fawaz Al-Hajji Aboud.2013. Effect of adding yeast Bread for two types of culture medium in production and concentration of some chemical compounds for shellfish. Iraqi Journal of Agricultural Sciences-44 (3): 397-403.
- Shimada, A., Kusano M., Takeuchi S., Fujioka S., Inokuchi T., Kimura Y.,.2002. "Aspterric acid and 6-hydroxymellein, inhibitors of pollen development in *Arabidopsis thaliana*, produced by *Aspergillus terreus* " J. of biosciences, 57 (5), pp. 459-464.
- Singer,R. 1986.The Agaricales in Modern Taxonomy (4th ed.). Koenigstein Königstein im Taunus, Germany: Koeltz Scientific Books., ISBN 3-87429-254-1.
- Stamets,P.,.2001. Novel antimicrobials from mushrooms. Turkey Tail on yunzhi.www. fungi.com / mycomed. html
- Stamets,P. 1993 Growing gourmet and medicinal mushroom Berkeley califonia. pp 544.
- Wang H., Lie Y., Yan L., Wan L., Ren X., Chen S., Dai X., Guo W. and Jiang Hand Liao B.,. 2016. "Functional Genomic Analysis of *Aspergillus flavus* Interacting with Resistant and Susceptible Peanut " MDPI J., 8(46): 1-16.
- Wood W.F. G.R. Farguar and D.L. Largent. 2000.Different volatile compounds from mycelium and sporocarp of *pleurotus ostreatus*. biochemical systematics and Ecology. 28:89-90.