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Biological control of some Pathogenic Fungi Isolated from Wastewater Treatment Plants using of Biocontrol Fungi

Etab Y. Allehebi [1], Nihad H. Mutlag [2], Ebtisam E. Ali [3], Asmaa A. Kadhim [4], Mohammed S. Jumaa [5] ,[6]Mohammed R.M
[1,3,4]Kufa University –Faculty of Education of Girls-Geography Department
[2,5,6]Kufa University –Faculty of Science-Ecology Department

Email: nuhadh.alazerjawi@uokufa.edu.iq

Abstract
This study aimed to evaluate the contribution of isolates of *Pleurotus ostreatus* and *Trichoderma harzianum* to the antagonistic activity of eight fungi isolated and identified from a wastewater treatment plant in Al-Najaf. Eight fungal isolates were identified based on their morphological features. These results indicate that the following fungi are common: *Acremonium sp., Alteraria alternatie, Aspergillus terrus, Aspergillus flavus, Aspergillus oryza, and Aspergillus caespitosis.* Through dual cultivation on Petri plates and PDA medium, this study demonstrated the significant antagonistic capability of *Pleurotus ostreatus* and *Trichoderma harzianum* against isolated filamentous and pathogenic fungi. It also showed a significant variance in the inhibitory spectrum of the biocontrol fungus against pathogenic fungi isolated from wastewater and effluents. Upon diagnosis, it was found to be at the (1 and 2) degrees on the five-degree Bell scale. Additionally, the weight of the fungal mass increased significantly after 21 days of cultivation of both harmful and biocontrol fungi. This suggests that *Pleurotus ostreatus* and *Trichoderma harzianum* both have significant antagonistic potential against filamentous and pathogenic fungi during dual growth on the PDB culture medium. There were also notable differences in the biomass weights. After 21 days of culture, the weights of *P. ostreatus* were 4.54, 4.69, 4.87, 4.88, 4.96, 5.12, 5.23, and 5.43 g, and 3.22 and 4.10 g for the control. *T. harzianum* generated eight isolates, and their weights were 3.55, 4.67, 4.77, 4.85, and 4.98 g respectively.
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Key Words: *Pleurotus ostreatus*, *Trichoderma harzianum*, antagonistic

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Introduction

Water comprises approximately 72% of the earth's surface and about 65% of the human body. Everyone wants access to clean water for personal use, drinking, and recreation. Water that is tainted loses its aesthetic and economic value and might potentially endanger the ecological systems (Metcalf *et al*, 2003).

Hazardous microorganisms like bacteria and Fungal contamination of water represents a potential environmental and public health concern and a top priority for both society and local authorities (Crini ,2005)

Water contamination occurs when anything like microbial pollution or something more negatively changes water. These may lead to real problems for the environment and the habitats of animals, as well as for humans and animals (Crini *et al*,2017). Microbial pollution is the main source of many types of wastewaters. There are some suspended, dissolved, and organic particles in its

composition of 1.0% solids and 99.9% water. Organic compounds constitute approximately 70% of the particles found in wastewater (Yassin *et al*,2018; Krantz *et al*,2005).

Fungi are eukaryotic microorganisms, such as molds and yeasts (Hawksworth *et al*,2017). Unlike bacteria, plants, and certain protozoa, fungi are categorized as belonging to a different kingdom because of the presence of chitin in their cell walls. These fungi are referred to as heterotrophic organisms because they obtain nourishment by releasing digestive enzymes into their environment and consuming the dissolved substances (Kuguk *et al*,2002).

Aquatic fungi, often referred to as water hyphomycetes, Ingoldian fungi, or amphibious fungi, represent a polyphyletic group of fungi. Definitions state that Aquatic fungi are a vast and diverse group of species that play a key role in maintaining aquatic ecosystems via the production of essential organic compounds, food web regulation, carbon and nutrient cycling, and energy transmission (Cairney,2005).

Phytopathogenic fungi have coexisted with aquatic plants since the beginning of their evolution. Despite being a natural phenomenon, their existence has sometimes had a detrimental effect on human health. Since the dawn of research, biomethods have been used to decrease the impact of phytopathogens in agricultural and aquatic settings. However, the subsequent integration of biological control with previously proven methods has further revolutionized the management of aquatic ecology and *treatment* plants (Mutlag *et al*,2021). Trichoderma spp. have recently been found to have potential broad use as BCAs against a range of aquatic and soil-borne phytopathogenic fungi (Hadar,1984). It is well known that the fungi *Trichoderma and Pleurotus ostreatus* have antagonistic effects on a range of fungi and bacteria, invertebrates, and other water-phytopathogens. *Pleurotus ostreatus*, also referred to as the oyster mushroom, oyster fungus, hiratake, or pearl oyster mushroom, is a common edible mushroom (Davidson *et al*,2014). Owing to their rapid generation time, high rates of asexual and sexual reproduction, and target specificity, fungi have greatly increased the potential applications of fungal biological control agents against pathogens.

The main factors influencing the activity of biocontrol agents (BCAs) include their physiology, aggressive growth pattern, and abundance of antimicrobial and antagonistic compounds that they produce. If *the BCA potential of Trichoderma spp.* is completely utilized, both indoor and outdoor plants, as well as agricultural crops, may flourish more quickly (Harman *et al*,2004).

The goal of this study was to combat pathogenic fungi that have been detected in the wastewater treatment plant in Al Brakia, Najaf, by using the antagonistic mechanisms of *the biocontrol fungus Pleurotus ostreatus and Trichoderma harzianum*.

1. To evaluate the antagonistic ability (Bell Scale) of two isolates of *Pleurotus ostreatus and Trichoderma harzianum* against eight pathogenic fungi isolated from wastewater on PDA medium, a double-culture investigation was conducted.
2. Evaluation of two isolates of *Pleurotus ostreatus and Trichoderma harzianum's* antagonistic ability against eight different pathogenic fungi isolated from wastewater on PDB medium.

Materials and Methods

Study area

The Al-Barakia wastewater treatment plant is located in the Najaf Governorate, southeast of Kufa, on the Euphrates River (Shatt al-Kufa), at coordinates (N 32 00 40, E 44 25 20). This location is shown in Figures 1&2. There are two distinct initiatives in the wastewater treatment plant. The most recent project, which consisted of the Compact and Bio_Sheft units as treatment units, provided the samples.

After treatment, the biomass of fungal growth was determined and contrasted with a control treatment that used only biocontrol fungi.

Although fungal communities are known to occur in wastewater treatment systems, their control has been studied far less than bacterial contamination. Specifically, the antagonistic potential of biocontrol fungi against pathogenic

fungal isolates from wastewater treatment plants remains insufficiently investigated.

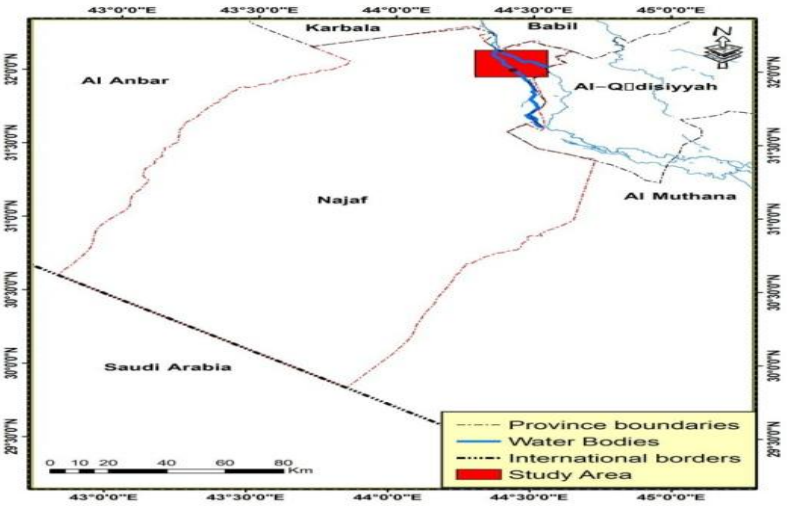


Figure 1: The map of study area of Albarakiya waste water treatment plant

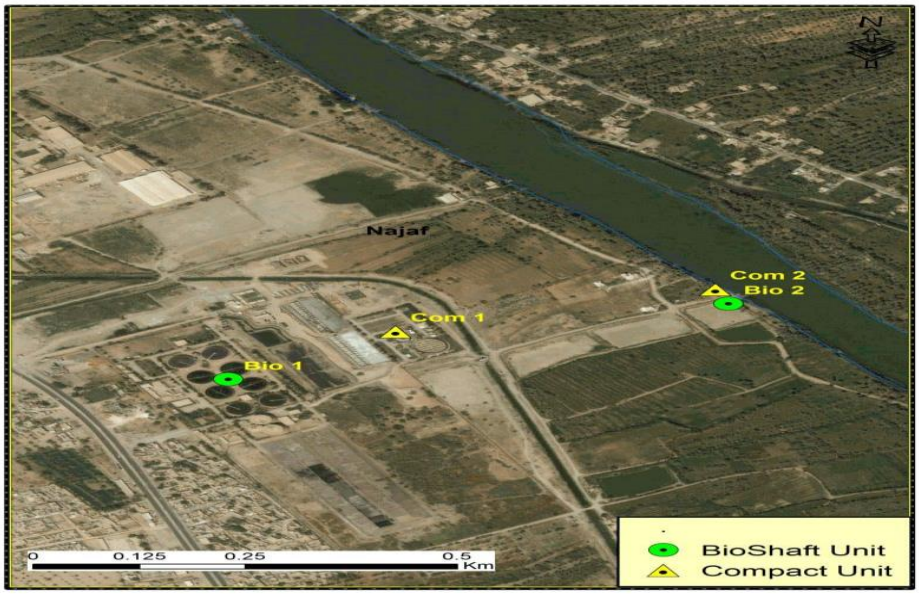


Figure 2: Al-Barakiya waste water treatment plant

Media Employed in Fungi Isolation Research

Potato dextrose Agar (PDA)

Potato dextrose Agar (PDA) was prepared according to Himedia method that used of potato dextrose agar, which was created for the isolation of fungi (Hawksworth *et al*,2014).

Potato Dextrose Broth (PDB)

Potato Dextrose Broth (PDB) was prepared according to Himedia method (Melo *et al*,2011).

The process of preparing the fungal filtrate

The fungal filtrate used in this study was prepared from PDB according to Melo *et al*,2011).

Calculate the growing biomass of *T. harzianum* and *Pleurotus ostreatus*

To determine the mass of *T. harzianum* and *Pleurotus ostreatus*, fungal growth was eliminated once the filter was acquired. The fungal biomass was then placed in a glass Petri dish, weighed, and oven-dried for 30 min at 85 °C. Lastly, the biomass was weighed to

calculate the weight differential (Ko *et al*,1970).

Antagonistic ability test between *T. harzianum* and *Pleurotus ostreatus* and fungi isolated from wastewater treatment plant on PDA media

The antagonistic ability of *T. harzianum* against *Pleurotus ostreatus* and the isolated fungi from wastewater was tested using the double culture method in a Petri dish containing sterile PDA medium (Bell *et al*,1982).

Result and Discussion

Reculturing of fungi isolated from wastewater treatment plants.

Eight isolates were selected for reculturing on PDA medium. They were then treated with biocontrol fungi (antagonistic mechanisms) using PDA and PDB media, and the inhibitionzonese on PDA and fungal biomass on PDB media were calculated.

Morphological identification of the isolated fungi

The fungi isolated in this study were identified based on their morphological characteristics, as described by Leslie *et al*.(2006). The phenotypic characteristics of the purified fungi after 5-7 days are shown in Table (1). Representative colony morphologies of the fungal isolates grown on PDA medium after 7 days of incubation are shown in Figure 3.

Table(1): Colony phenotypic characteristics of fungi isolated from the wastewater treatment plant.

Species	Colony diameter	Colony color
A. flavus	Grater than 400µm	Yellowish-green
A.niger	67-70µm	Black to dark brown
A.tubingensis	65-72µm	Black
A. alternate	_	Dark green to black
***Acremonium* sp**	1-3 cm	Light green/white
A. terreus	500×30-50 µm	Brownish
A.caespitosus	2.0-2.5µm	Brown
A.oryzae	60-75mm	pale yellow

Figure 3. Morphological characteristics of fungal isolates from the wastewater treatment plant.

Trichoderma harzianum *Pleurotus ostreatus*

Figure (4)*:* Morphology of *biocontrol fungi* used in the study.

Calculating the growing biomass of *T. harzianum and Pleurotus ostreatus* on PDB media

The results showed a significant increase in the biomass of (*T. harzianum*) and (*P. ostreatus*) compared to the control group on PDB medium. Table (2) represents the weights of the live mass of *T. harzianum and P. ostreatus* after 21 days of incubation with fungi isolated from wastewater. These findings suggest that fungi can be biocontrol agents through antagonism against pathogenic fungi isolated from wastewater.

Table (3) shows that *T harzianum* gave a significantly higher biomass weight with *A. niger* isolate that reached 4.98 g in comparison with the control of *T harzianum* isolate, which was 3.82 g, while the lowest biomass weight was with *Acremonium sp,* which was not significantly at 3.99 g.

In addition, *Pleurotus ostreatus* bioconrtol fungi gave significant weights as afinal weights after 21 days of incubation with isolated fungi from waste water plant (Table 3& Figure5), which illustrated the significant weight with *A. tubingensis* (4.96 g) and the lowest weight with *A. terreus (*4.54 g).

Table (2): Final biomass weights. *T harzianum* was incubated with fungal isolates from the wastewater treatment plant.

NO A	***T.harzianum* with**	**Final weight g**
1	*Acremonium* sp	3.99
2	*A. alternate*	4.23
3	*A.terreus*	4.85
4	*A. flavus*	4.12
5	*A.oryzae*	4.77
6	*A.caespitosus*	4.67
7	*A.tubingensis*	4.55
8	*A.niger*	4.98
Control	Only *T.harzianum*	3.82
LSD		0.284

Table (3): Final biomass weights. *P. oustreatus* incubated with fungi isolates from wastewater treatment plant.

NO B	***P.ostreatus* with**	**Final weight gm**
1	*Acremonium* sp	5.12
2	*A. alternate*	4.88
3	*A.terreus*	4.54
4	*A. flavus*	5.43
5	*A.oryzae*	4.69
6	*A.caespitosus*	4.87
7	*A.tubingensis*	4.96
8	*A.niger*	5.23
Control	Only *P.ostreatus*	4.50
LSD		0.253

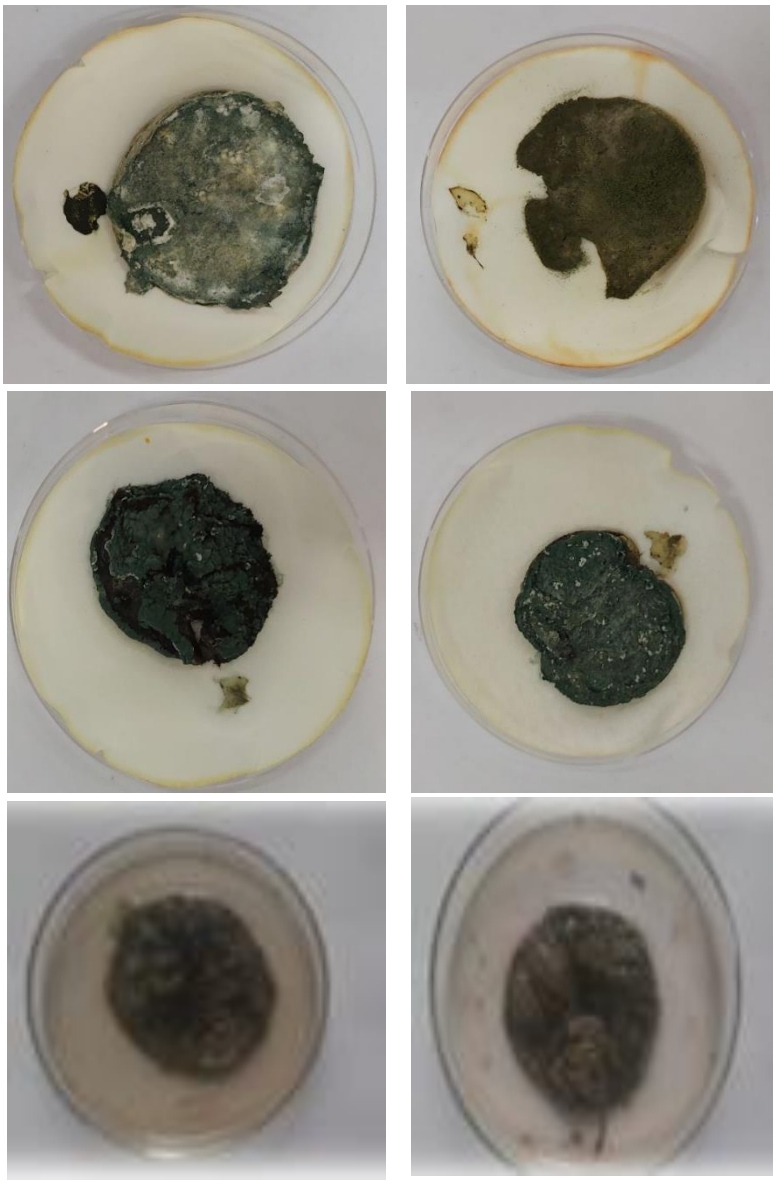


Figure (5): Biomass production of biocontrol fungi in dual culture.

The results of the study showed an increase in the growth of fungi used in the Al-Barakia wastewater treatment plant, and the reason for the increase in weight was attributed to the ability of fungi to biocontrol other isolates by antagonistic mechanisms through cracking the hyphae and mycelium of pathogenic fungi (Papagianni *et al*,2004).

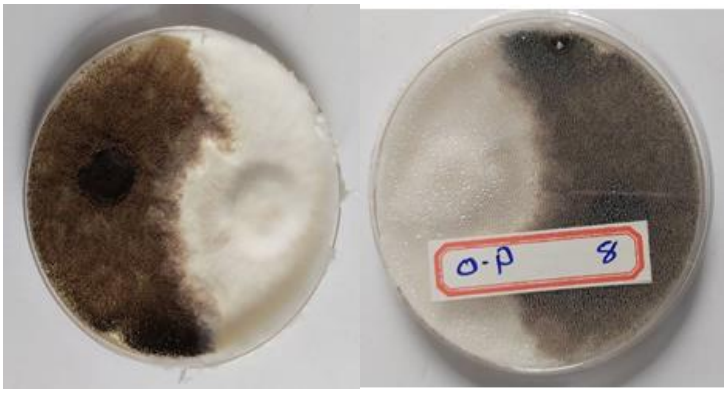
Antagonistic ability test between *T. harzianum* and *Pleurotus ostreatus* and fungi isolated from wastewater treatment plant on PDA media

The results of the investigation showed that *P. oustreastus and T. harzianum* could antagonistically achieve the first, second, and third degrees of the bell scale, respectively, against all isolates from wastewater plants that were evaluated (Figure 6).

According to this study, *P. oustreastus and T. harzianum* are important for biological control and may have the ability to prevent pathogens. During this time, research has been conducted, leading to the identification of the mechanisms of action and their many uses. In double cultures, the pathogen's inhibition grew rapidly as soon as the antigen came into contact with it. *Phaloderma and Trichoderma* spp. exert antagonistic effects when their hyphae surround and damage the cell wall. This parasitic impact prevents harmful fungi from growing and becoming active.

The results of the investigation agreed with (Chaudhry *et al*,1999), which claims that *T. harzianum* and *P. oustreastus* strongly inhibits *A. flavus* as well as hazardous fungi. The strong antifungal activity of *T. harzianum* is positively correlated with its ability to produce antifungal compounds (alkyl pyrones), such as butenolide and harzianolide, which inhibit the growth of other fungi. Furthermore, some studies have connected *T. harzianum and P. ostreatus* to the production of lytic enzymes, which degrade the toxin components of the fungal cell wall and allow wedges to pass through, rupturing cell components and finally causing death (. Roco et *al*,2001). *T. harzianum* was shown to produce β-glucosidase and cellulase after 24 hours of growth. These enzymes produced by pathogenic fungi were inactivated and contributed to hostile behavior in dual cultures. The study further confirmed *the ability of T. harzianum* to block the first degree by showing that it eradicated *T. asperellum* (De Marco *et al*,2003).

T. harzianum and *P. oustreastus* were classified as first degree since it completely prevented *A. alternata* from developing and develops more quickly and unconstrained in diploid cultures. Because of its rapid development, *Trichoderma* has a significant advantage over pathogens in the struggle for resources and space; a Petri dish containing *T. harzianum* is brimming with them (Mirkova,1982).




O·P 7
O·P 6
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O·P 4

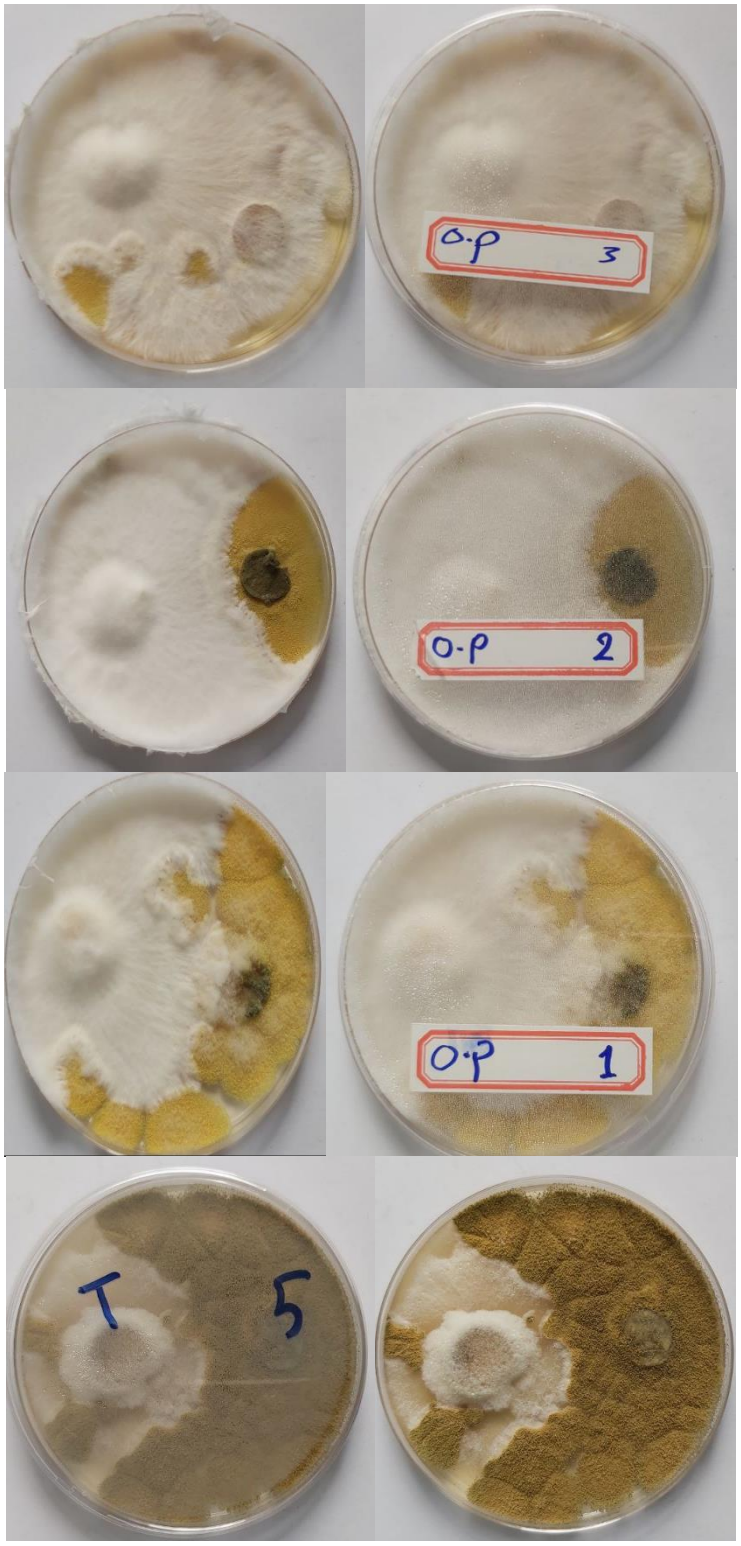
O.P 3
O.P 2
O.P 1
T 5

Figure 6: Antagonistic interaction between biocontrol fungi and pathogenic fungal isolates.

Conclusion

With eight well-chosen isolates from wastewater on PDA, *Trichoderma harzianum* has great potential to provide a considerable inhibitory zone. With eight well-chosen isolates from wastewater on PDA, *Pleurotus ostreatus* has great potential to provide a considerable inhibitory zone. With eight well-

chosen isolates from wastewater on PDB, *Trichoderma harzianum* has a strong potential to provide considerable biomass weight. With eight well-chosen isolates from wastewater on PDB, *Pleurotus ostreatus* has a great capacity to yield a notable biomass weight. The genera Trichoderma harzianum and Pleurotus ostreatus have a high antagonistic potential against filamentous and pathogenic fungi.

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المعالجة البيئية لبعض الفطريات الممرضة المعزولة من محطات معالجة مياه الصرف الصحي باستخدام بعض فطريات المكافحة الحيوية

عتاب يوسف اللهيبي [1]، نهاد حبيب. مطلّك [2]، ابتسام إبراهيم. علي [3]، أسماء علي. كاظم [4]، محمد جمعة [5]، ، محمد رياض[6].

[1، 3، 4] جامعة الكوفة - كلية التربية للبنات - قسم الجغرافيا

[2، 5، 6] جامعة الكوفة - كلية العلوم - قسم البيئة

nuhadh.alazerjawi@uokufa.edu.iq

المستخلص

هدفت هذه الدراسة إلى تقييم مساهمة عزلات فطر المحار *Pleurotus ostreatus* وفطر التريكوديرما هارزيانوم *Trichoderma harzianum* في النشاط المضاد لثمانية أنواع من الفطريات التي تم عزلها وتحديدها من محطة معالجة مياه الصرف الصحي في النجف. تم تحديد ثمانية عزلات فطرية بناء على خصائصها الشكلية. وتشير النتائج إلى أن الفطريات التالية كانت شائعة: *Acremonium sp*، *Alteraria alternata*، *Aspergillus terreus*، *Aspergillus flavus*، *Aspergillus oryza*، *Aspergillus caespitosis*، *Aspergillus tubingesis*، و*Aspergillus niger*. من خلال الزراعة المزدوجة على أطباق بتري ووسط PDA، أثبتت هذه الدراسة القدرة المضادة الكبيرة لفطر المحار وفطر التريكوديرما هارزيانوم ضد الفطريات الخيطية المعزولة والفطريات الممرضة. أظهرت الدراسة أيضًا تباينًا ملحوظًا في نطاق تثبيط الفطر المُستخدم في المكافحة الحيوية للفطريات الممرضة المعزولة من مياه الصرف الصحي والمياه العادمة. عند التشخيص، وُجد أن الفطر يقع ضمن الدرجتين الأولى والثانية على مقياس بيل ذي الخمس درجات. بالإضافة إلى ذلك، ازداد وزن الكتلة الفطرية بشكل ملحوظ بعد 21 يومًا من زراعة كل من الفطريات الضارة والفطريات المُستخدمة في المكافحة الحيوية. يشير هذا إلى أن فطر المحاري *Pleurotus ostreatus* وفطر التريكوديرما هارزيانوم *Trichoderma harzianum* يتمتعان بقدرة مضادة كبيرة ضد الفطريات الخيطية والفطريات الممرضة عند زراعتهما معًا على وسط زراعة PDB. ولوحظت اختلافات ملحوظة في أوزان الكتلة الحيوية أيضًا. فبعد 21 يومًا من الزراعة، بلغت أوزان فطر المحار 4.54، 4.69، 4.87، 4.88، 4.96، 5.12، 5.23، و5.43 غرامًا، بينما بلغت 3.22، 4.10 غرامًا للمجموعة الضابطة. أنتجت فطرية التريكوديرما هارزيانوم ثماني عزلات، وكانت أوزانها 3.55، 4.67، 4.77، 4.85، و4.98 غرام على التوالي.

الكلمات المفتاحية: الفطر المحاري Pleurotus ostreatus، التريكوديرما هارزيانوم، مضاد، ممرض.