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ANTIFUNGAL ACTIVITY OF GARLIC AND NEEM EXTRACTS AGAINST COBWEB MOLD DACTYLIUM AND GREEN ROT DISEASES IN *Agaricus bisporus*

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ABSTRACT

The study was conducted to control cobweb disease caused by *Cladobotryum dendroides* and green mold disease caused by *Trichoderma aggressivum*, which infect the white fungus *Agaricus bisporus* crop. The laboratory results on the pathogenicity of the two pathogenic fungi showed their ability to cause infection in the white fungus and the emergence of symptoms. The addition of garlic and neem extracts, separately, at three concentrations (0.25, 0.5, and 1%) to the media, potato dextrose agar (PDA), led to a significant decrease in the percentage of inhibition of pathogenic fungi *C. dendroides* and *T. aggressivum*. The highest inhibition rates obtained when using garlic extract at a concentration of 1% against the two pathogenic fungi amounted to 62.93 and 74.97%, respectively, while the name extract recorded the highest inhibition rate at the same concentration, reaching 43.47 and 32.37%, respectively. Also, when testing the extracts in the traditional cultivation of white fungus, it was noted that the garlic extract at a concentration of 1% was significantly superior in controlling infection, and no symptoms appeared when using it, the use of plant extracts at different concentrations did not record a significant difference in the period required for the emergence of fungal pins compared to the control treatment without a pathogen, the highest amount of white fungus production was 1000 g/bag when using garlic extract at a concentration of 0.5% in the presence of the pathogen *C. dendroides*, while the highest amount of production was 1355.0 g/bag when using neem extract at a concentration of 0.25% when infected with the pathogen *T. aggressivum*.

Keywords: *Agaricus bisporus, Cladobotryum dendroides, Trichoderma aggressivum.*

تأثير مستخلصي الثوم والنيم في مكافحة مسبب مرض نسيج العنكبوت والعفن الاخضر في محصول الفطر الابيض *Agaricus bisporus*

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الخلاصة:

أجريت الدراسة للسيطرة على مرضي نسيج العنكبوت (الكلادوبوتريوم) مسببه الفطر *Cladobotryum dendroides* ومرض العفن الاخضر ومسببه الفطر *Trichoderma aggressivum* اللذين يصيبا محصول الفطر الابيض *Agaricus bisporus*، اظهرت النتائج المختبرية للمقدرة الإمراضية للفطرين الممرضين قدرتهما على احداث

[*] **The research is taken from a master's thesis by the third researcher.**

الاصابة للفطر الابيض وظهور الأعراض، أوضحت النتائج أن إضافة مستخلصي نباتي الثوم والنيم منفصلين وبثلاثة تراكيز (0.25 و 0.5 و 1 %) لكل منهما الى الوسط الزرعي مستخلص البطاطا والدكستروز الصلب أحدث انخفاضاً معنوياً في نسبة تثبيط الفطريات الممرضة *C . dendroides* و *T . aggressivum* وكانت أعلى نسبة تثبيط حصلت عند استخدام مستخلص الثوم بالتركيز (1%) بلغ 62.93 و 74.97% ضد الفطرين الممرضين وعلى التوالي، في حين سجل مستخلص النيم أعلى نسبة تثبيط بلغت 43.47 و 32.37 % لهما في التركيز ذاته وكذلك الحال عند اختبار المستخلصات في الزراعة التقليدية للفطر بتفوق معنوي لمستخلص الثوم بتركيز 1% في كبح الاصابة ولم تظهر أي أعراض عند أستخدامها، لم يسجل استخدام المستخلصات النباتية بمختلف التراكيز فرقًا معنويا يذكر في الفترة الازمة لظهور الدبابيس عن معاملة السيطرة بدون ممرض فيما سجلت أعلى كمية انتاج للفطر الابيض بلغت 1000غم/ كيس بوجود الفطر الممرض *C . dendroides* عند استخدام مستخلص الثوم بتركيز 0.5 % فيما كانت أعلى كمية انتاج بلغت 1355.0 غم/ كيس عند الإصابة بالفطر *T . aggressivum* عند استخدام مستخلص النيم بالتركيز 0.25%.

الكلمات المفتاحية: الفطر الابيض، مرض نسيج العنكبوت، مرض العفن الاخضر.

INTRODUCTION

The white mushroom, *Agaricus bisporus*, is distinguished from other edible species due to its large fruiting bodies, in addition to its delicious taste and distinctive flavor when cooked, not only for its nutritional benefits but also for its health benefits **(Chang & Wasser, 2017; Ibrahim *et al*., 2021).** The cultivation of white mushrooms is exposed to many problems and pests; perhaps its most important are fungal diseases that occur at various stages of production and lead to a lack of production of fruiting bodies or a decrease in their qualitative characteristics **(Nathaniel *et al*., 2020).** The white fungus is infected with several pathogens; perhaps its most important is *Cladobotryum*, the pathogen of the cobweb mold dactylium disease, and green molds, which include several types of *Trichoderma* spp. that cause indirect losses by attacking the mycelia **(Kosanovic *et al*., 2013; Chakwiya *et al*., 2019).** The great and increasing interest of most researchers in the use of medicinal plants and the identification of their products and the active substances in these plants to control pathogens in order to reduce the use of chemical pesticides and solve the problems resulting from excessive use of them in order to obtain healthy food and free of pesticide residues, especially in the cultivation of mushrooms, due to its peculiarity, where the age of the fruiting bodies does not exceed one week in the development hall, so this study was carried out to test the natural plant extracts (garlic and neem) to control cobweb and green mold diseases, as they are safer and faster to decompose **(Kumar *et al*., 2008; Siripornvisal *et al*., 2009).**

Four fungal pathogens were recorded in white fungus farms (*A. bisporus*) in Iraq for the first time. Three of them directly attack the fruiting bodies, such as cobweb mold dactylium disease, caused by the fungus *Cladobotryum dendroides*; dry bubble disease caused by *Lecanicillium fungicola*; wet bubble disease caused by *Mycogone perniciosa*, and the fourth is a pathogenic fungus that causes green rot, which includes several species of *Trichoderma* spp. that cause indirect losses by attacking the mycelium **(Hassan, 2013).**

The first attempt to grow the fungus *A. bisporus* was conducted in the eighties of the last century at Al-Hamidiya farm in Al-Anbar Governorate in Iraq to support the local market with a small part of the production of white mushrooms **(Hassan, 2013)**, and according to the information available in the Ministry of Agriculture, Plant Protection Department, Department of Organic Agriculture, Project of Organic Fertilizer Preparation, and Mushroom Cultivation, it is responsible for following up the mushroom farms in Iraq and granting licenses to establish the mushroom farms. There are four farms in Iraq, namely Al-Wadq farm in Baghdad Governorate, Al-Wahda district, with a production capacity of 500 tons annually, and three farms in the Kurdistan region with the same production capacity, in addition to the presence of

small farms throughout Iraq, such as Al-Hamdaniya farm in Mosul and Al-Diwaniya farm, with a production capacity of 125 tons annually.

The researchers indicated the use of various safe methods to reduce the incidence of various diseases and their resistance to increase production, especially the use of environmentally friendly pesticides prepared from plant extracts to reduce the use of chemical pesticides that have negative effects on humans, their health, the ecosystem, and residues in the fruiting bodies **(Chakraborty & Archana, 2021).**

MATERIALS AND METHODS

Isolation and diagnosis

The covered soil samples and culture medium were collected from several sites for growing the white fungus *Agaricus bisporus* in Iraq, where infection with the pathogenic fungus appeared. Three isolates of the fungus *Cladobotryum* spp. and two isolates of the fungus *Trichoderma* sp. were diagnosed to the level of the genus phenotypically, depending on the shape and color of the colony, the shape of the conidiophore, and the spores formed using the taxonomic keys adopted in the Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad **(Hatvani *et al.*, 2008; Prameeladevi *et al.*, 2018).** The two most pathogenic species were molecularly identified, and the nucleotide sequences obtained from the NCBI GenBank were deposited under the accession numbers of T*richoderma aggressivum* (OQ109172) and *Cladobotryum dendroides* (OQ048864).

Testing the pathogenicity of pathogenic fungi in the laboratory

Healthy fruiting bodies belonging to the white fungus (*Agaricus bisporus*) were obtained from the local markets, then the fruits were cut into two halves and placed in petri dishes. A disc of 0.5 mm diameter was taken from each isolate of the two fungi, *Trichoderma* spp. and *Cladobotryum* spp., by using a cork puncture, and it was placed on the fruiting body in two locations, on the cap and the stem, then incubated at a two temperature of 24 and 4 C^o **(Lakkireddy *et al.*, 2020)**, after which readings were taken and the percentage of infection severity of the fruiting body was measured according to the pathological evidence.
0 = without symptoms, 1 = 1 - 10%, 2 = 11 - 25%, 3 = 26 - 50%, 4 = 51 - 75%, and 5 = < 75% by applying the **Mckinney equation (1923).**

Molecular diagnosis of the two pathogenic fungi *Cladobotryum dendroides* and *Trichoderma aggressivum* by Polymerase Chain Reaction (PCR) technique.

After the initial diagnosis of the two pathogenic fungi, *C. dendroides* and *T. aggressivum*, the diagnosis was confirmed using the polymerase chain reaction Polymerase Chain Reaction **(**PCR) method. The isolate that gave the highest pathogenicity to mushroom fruits was diagnosed in vitro **(Lakkireddy *et al.*, 2020).**

The isolates of *C. dendroides* and *T. aggressivum* were active on Potato Dextrose Agar (PDA) by the single spore method and incubated in the incubator at a temperature of 25 ± 2 C^o for seven days. After completing the growth of the fungal colony of the two fungi, they were sent to the Musayyib Bridge Company in order to conduct the DNA extraction stages from *C. dendroides* and *T. aggressivum*. The DNA extraction process was carried out using Plant Kit, a commercial kit prepared by the Korean company Bioneer, following the approved steps of the company.

DNA replication

Five microliters of extracted nucleic acid and one microliter of ITS4 primer were added with 11 microliters of ionic distilled water (Table 1), and placed in small tubes 0.2 ml containing 5 microliters of Master Mix, and the materials were mixed by shaker, then centrifuged for 15 seconds with shaking for 5 seconds, then the tubes were transferred to a thermal polymerization PCR device under optimal conditions for cycles (Table 2) (**White *et al.*, 1990**), and the DNA pieces that were doubled were sent to the South Korean company (Macrogen Inc.) for the purpose of nucleotide sequence determination.

Table (1): The primers used in the polymerase chain reaction

The sequence of nitrogenous bases	Primer
3 -TCCGTAGGTGAACCTGCGG -5	ITS1
3-TCCTCCGCTTGATATGGC-5	ITS4

Table (2): Thermal polymerization program to amplify the DNA extracted from the fungus *T. agressivum*.

Cycle / Repeat	Time	Temperature c°	PCR s Step
1	5 min	95 c°	Pre- Denaturation
25-35	30 sec	95 c°	Denaturation
	30 sec	55 c°	Annealing
	1min	72 c°	Extension
1	min5	72 c°	Final extension

Gel Electrophoresis

The electrophoresis technique was adopted using the acarose gel (1%) to detect DNA bunds, and the acarose gel was prepared by melting 1 g of acarose in 90 ml of sterile distilled water and 10 ml of solution (10x TBE Buffer), and the mixture was heated with the Microwav device for 3 minutes, then cool the mixture and add 5 microliters of ethidium bromide, and mix the mixture with light shaking, and then the acarose gel template was prepared. The sterile comb by ultraviolet rays (UV) rays was put at one end of the template to make holes in the gel, then poured the acarose gel and left it to cool and harden for 30-45 minutes at the laboratory temperature, after which the comb was removed and 5 microliters added to it from the DNA pieces (Ladder) to the first hole (containing the pieces of the standard DNA and adding the same amount of the extracted DNA to the other holes and electrophoresis was done at 70 volts for a period of 60 minutes, to detect the extracted and amplified DNA bunds, which represents PCR products. The DNA pieces were purified and samples were sent to the Korean company (Bioneer) in order to determine the sequence of nitrogenous bases.

Evaluating the effectiveness of plant extracts in inhibiting the pathogenic fungi and white fungus

A solution of oily neem extract and alcoholic garlic extract with concentrations (0.25, 0.5, and 1%) was prepared with PDA, then poured separately into petri plates (9 cm). After the medium became solid, agar discs (5 mm) of pathogenic fungi and white fungus were transferred to a petri plate in their centers by using a cork borer, then incubated at 25±1 C^o at five days for the pathogenic fungi and 21 days for the white fungus. The mean diameter of

fungus growth was measured, and the inhibition ratio was calculated according to the following formula **(Montealegre *et al*., 2003).**

% Inhibition = (1- control colony/treatment colony) X 100

Evaluation of pesticide efficacy in the growth of the two pathogenic fungi *C. dendroides* and *T. aggressivum* and the white fungus *A. bisporus* in a laboratory

Avistin suspension FL and its active substance, carbendazim 50% SC, were used. A solution of the pesticide Bavistin was prepared at the recommended concentration (2.5 ml / liter) and added to the culture medium Potato Dextrose Agar (PDA), then it was poured into petri dishes (9 cm) and left until it hardened and the dishes were inoculated with pathogenic fungi *C. dendroides* and *T. aggressivum* in its center using a 5 mm cork borer separately and placed in an incubator at a temperature of 25 ± 2 C^o for 5 days for the two pathogenic fungi and 21 days of incubation for the white fungus *A. bisporus* until the completion of the growth of the control treatment, the results were taken by measuring two orthogonal diameters for each dish and the percentage of inhibition was calculated according to the equation used in the previous experiment.

Preparation of the pathogenic fungus inoculum

The fungi inoculum was prepared by placing an agar disc (5 mm) from the seven-day-old culture by cork borer on the middle of the PDA, and after the completion of the growth, 10 ml of sterilized distilled water was added for each dish, and the spores were scraped by a sterile metal rod for harvesting. The suspensions of the two fungi were collected separately in two sterile glass flasks (100 ml each). The dilution process was performed up to the 10^{-6} concentration using the hemocytometer chip.

The effect of adding garlic and neem extracts on the pathogenic fungi

After the process of planting and covering the white fungus, the infection was conducted by spraying the inoculum of the pathogenic fungi *Trichoderma* spp. and *Cladobotryum* spp. on the coverage soil at 100 ml per bag and leaving it for 24 hours to give it a period in order to germinate its spores, then spraying the garlic and neem extracts each with their concentration (0.25, 0.5, and 1%) on the coverage layer by 150 ml per bag and by four replicates for each treatment, after 10 days, the spraying was repeated on the coverage soil,

The infection percentage of the fruit bodies was estimated according to the following equation:

Percentage of infection % = the number of infected fruits/ total number x 100

The severity of infection from the pathogenic fungi was estimated at according to the pathological evidence.

0 = without symptoms, 1 = 1 - 10%, 2 = 11 - 25%, 3 = 26 - 50%, 4 = 51 - 75%, and 5 = < 75% by applying the **Mckinney equation (1923).** The necessary period for the appearance of fruit bodies and the amount of production for each treatment was determined by the weight of mature fruit bodies resulting from the multiple harvesting of white mushrooms, and the harvesting process continued for 30 days.

RESULTS AND DISCUSSION

Testing the pathogenicity of pathogenic fungi in the laboratory

The results in (Table, 3) showed that all tested fungal isolates at temperatures of 25 C^o and 4 C^o were effective in their pathogenicity on the fruiting bodies of the white fungus *A. bisporus*, which turned to a light brown color after five days of the inoculation. All isolates of *Cladobotryum* spp. had the highest infection severity at a temperature of 25 C^o, which ranged

between 72, 71, and 72% for the three tested isolates, respectively, compared to the control treatment, which recorded an infection severity of 0.0%. The isolates of *Trichoderma* spp. at a temperature of 25°C recorded an infection severity of 60% for each of the two tested isolates, while the above-mentioned isolates showed less damage to the white fungus at a temperature of 4 C^{o}. These results are consistent with previous studies, which showed that the pathogens that affect the fruiting bodies have a high pathogenicity that works to absorb nutrients from them, causing them to be small in size, change color, have low weight, and be completely damaged **(Lakkireddy *et al*., 2020).** In addition, these pathogens secrete wall-dissolving enzymes such as cellulases, chitinases, and glucanases that analyze cell walls, as well as hydrolytic enzymes that analyze proteins and turn them into amino acids **(Jayalal & Adikaram, 2007; Choi *et al*., 2018).**

Table (3): Pathogenicity test of some isolates of *Cladobotryum* spp. and *Trichoderma* spp. on the fruits of the white fungus *A. bisporus*

The mushroom	Treatments	Temperature 25 °C	Temperature 4 °C
	Control (only fruiting bodies)	0.0	0.0
Cladobotryum spp.	Isolation 1	72.0	60.0
	Isolation 2	71.0	60.0
	Isolation 3	72.0	55.0
Trichoderma spp.	Isolation 1	60.0	40.0
	Isolation 2	60.0	50.0
	LSD 5%	5.45	7.49

Molecular diagnosis of *Trichoderma aggressivum*

The results showed that the extraction of the total nucleic acid of the pathogenic fungus *T. aggressivum* under study and the presence of one bund of the pathogen fungus with a molecular weight of approximately 600 bp for *T. aggressivum*, which was identical to the phenotypic diagnosis (Fig, 1).

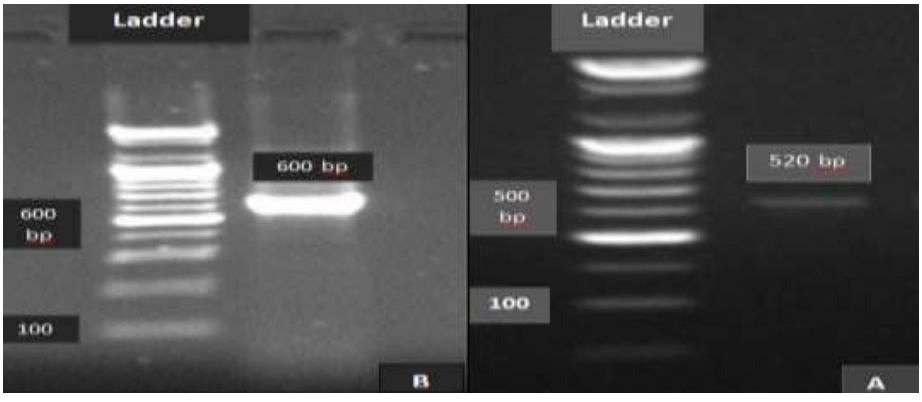

Fig (1): Electrophoresis of double-stranded DNA segments from the genomes of *C. dendroides* and *T. aggressivum* (A = *C. dendroides* / B = *T. aggressivum*) with a molecular weight of 520 bp and 600 bp base pairs, respectively, for the ITS region. Ladder DNA standard 100 base pairs

Reading the nucleotide sequences of the *Trichoderma aggressivum* DNA

The result of the nucleotide sequence of the fungus *T. aggressivum* after analysis compared with the GenBank was a high match with the pathogenic fungus *T. aggressivum*, which amounted to 99% with the global isolates in the International GenBank National Center for Biotechnology Information (NCBI). The nucleotide sequence of the Iraqi isolates under study was deposited in the GenBank under the accession number (Table 4), where the diagnosis of *T. aggressivum* was the first time in Iraq on the fruits and media of the white fungus *A. bisporus.*

Table (4): The accession numbers of Iraqi isolates of *C. dendroides* and *T. aggressivum*

Accession number	The isolate	
OQ048864	*C. dendroides*	1
OQ109172	*T . aggressivum*	2

It was found through the genetic affinity tree drawn by the SDTv1 program and by the method of neighborhood inclusion of the ITS region of the fungus *T. aggressivum* isolated from the fruiting bodies and the culture medium of the white fungus *A. bisporus* in Iraq with its equivalent counterparts from the gene bank, which recorded the highest match rate of 99%. The Iraqi isolate was lined up in one group with the international isolates, and *Rhizoctonia solani* was considered a fungus outside the group, as in Figure (2). The Iraqi isolate of the pathogen *T. aggressivum* was 99% identical to its counterpart isolates in Turkey (MH185822), South Africa (KX379163), Iran (MZ778797), and the Czech Republic (FN549908), depending on the molecular diagnosis and nitrogen base sequence of the Iraqi isolate. It is possible to adopt the classification of the pathogenic fungus *T. aggressivum*, which is isolated from the fruiting bodies, and the culture medium of the white fungus *A. bisporus*, which shows symptoms of infection with the pathogenic fungus *T. aggressivum*.

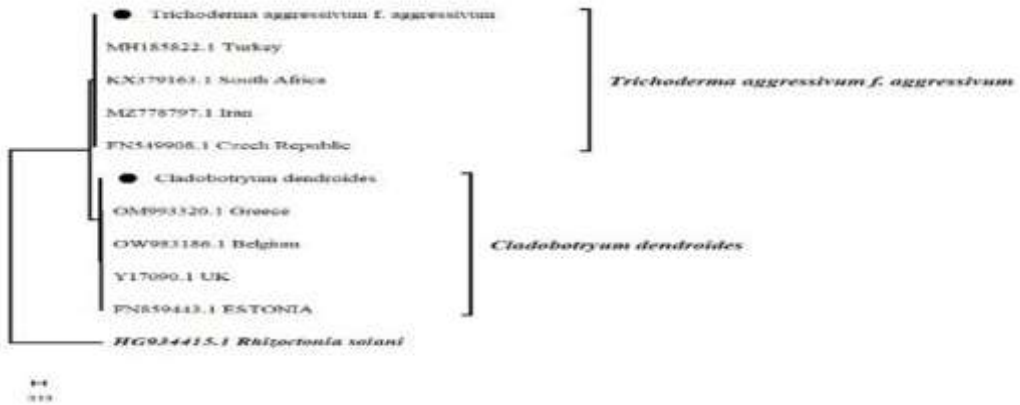

Fig (2): Genetic relationships of the two fungi *T. aggressivum* and *C. dendroides*

The genetic origins tree of neighbor joining was constructed from the nucleotide molecular sequence of the ITS region of the two isolates of the two pathogenic fungi *C.*

dendroides and *T. aggressivum* isolated from the fruits and culture media of the white fungus *A. bisporus* from the provinces of Baghdad, Babylon, and Diwaniyah, respectively (indicated by the symbol *) with their equivalent counterparts from the GenBank. The nucleotide sequence of the fungus *Rhizoctonia solani* was included for the purpose of comparison. Nucleotide analyses were performed using the MEGA11 program **(Muhire *et al*., 2014; Tamura *et al*., 2021).**

Evaluating the effectiveness of garlic and neem extracts on the growth of the fungi *Cladobotryum* spp., *Trichoderma* spp., and *A. bisporus*

The results in (Table, 5) and Figures (3 and 4) showed that the different concentrations had a variable effect on inhibiting the growth of pathogenic fungi. In general, there is an increase in the inhibition percentage with the increase in the extract concentration. The concentration (1%) of garlic and neem extracts gave the highest percentage of inhibition in the pathogenic fungi *C. dendroides* and *T. aggressivum*, which reached (62.93, 74.97%) and (43.47, 32.37%), respectively, compared to the control treatment, which recorded 0% in the inhibition percentage. The extracts also affected the white fungus in the laboratory, so the inhibition percentage at a concentration of 1% was 14.7 and 6.4%, respectively. The results were similar to those found by **Muhammad *et al*. (2019)** when using several plant extracts against *Cladobotryum mycophilum*. The effectiveness of garlic extract in inhibiting pathogenic fungi in the laboratory is attributed to its high content of effective compounds, especially allicin, which is an inhibitor against the growth of pathogenic fungi. In addition, it contains many chemical compounds such as saponins, flavonoids, alkaloids, and anthraquinones **(Francois, 1994; Singh & Divedi, 1999; Sarfraz *et al*., 2020; Sherifat *et al*., 2020).**

Table (5): Evaluating the effectiveness of garlic and neem extracts in inhibiting the fungi *Cladobotryum* spp., *Trichoderma* spp., and *A. bisporus*

Treatments	Inhibiting %			
	Concentration%	*C. dendroides*	*T. aggressivum*	*A. bisporus*
Control	0	0.0	0.0	0.0
Garlic extracts	0.25	0.0	0.0	7.1
	0.5	43.47	62.93	12.0
	1	62.93	74.97	14.7
Neem extracts	0.25	0.0	0.0	0.0
	0.5	38.43	29.2	2.8
	1	43.47	32.37	6.4
	LSD 5%	2.77	3.38	2.49

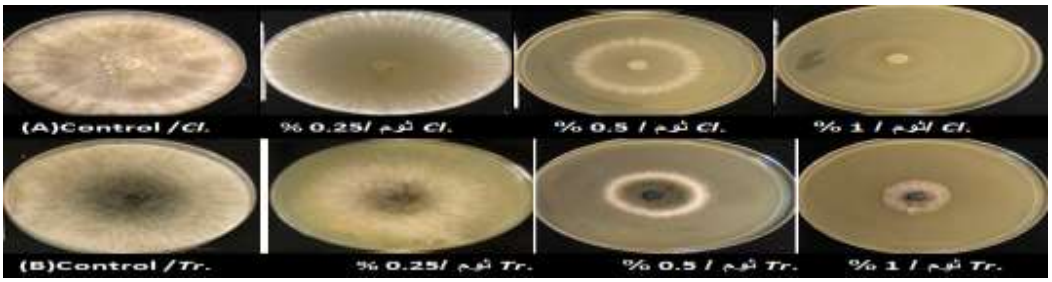

Figure (3): Evaluating the effectiveness of garlic extract in inhibiting the fungi *Cladobotryum* spp., *Trichoderma* spp., and *A. bisporus* Garlic + *C. dendroides* and (B) Garlic + *T. agressivum*

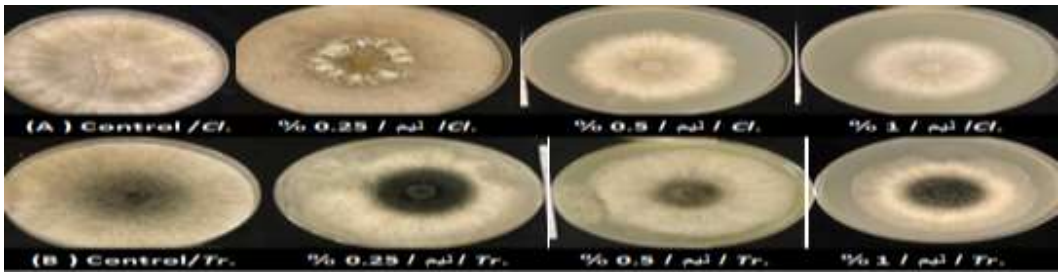

Figure (4): Evaluating the effectiveness of neem extract in inhibiting the fungi *Cladobotryum* spp., *Trichoderma* spp., and *A. bisporus* (A) Neem + *C. dendroides* and (B) Neem + *T. agressivum*

Evaluation of the pesticide effectiveness on the growth of the two pathogenic fungi *C. dendroides* and *T. aggressivum* and the white fungus *A. bisporus* in the laboratory

The results in (Table, 6) showed the effect of adding bavistin at the recommended concentration of 2.5 ml/L against the two pathogenic fungi *C. dendroides* and *T. aggressivum* and the white fungus *A. bisporus*. The pesticide recorded the highest inhibition rate against *C. dendroides* and *T. aggressivum*, reaching 75.90% and 68.47%, respectively, while it gave a low inhibition rate against the white fungus *A. bisporus*, amounting to 27.73%, compared to the control 0%, and these results agreed with **Chakraborty *et al*. (2013)**, who reported using bavistin with a concentration of 1.5% against the pathogenic fungus *T. hamatum* and *T. viride*, giving an inhibition rate of 97%.

Table (6): Evaluation of the pesticide effectiveness on the growth of the two pathogenic fungi *C. dendroides* and *T. aggressivum* and the white fungus *A. bisporus* in the laboratory

Treatments	Concentration	Inhibition %		
		T. aggressivum	*C. dendroides*	*A. bisporus*
Control		0.00	0.00	0.00
Bavistin	2.5 ml \ L	68.47	75.90	27.73
LSD 5%		23.81	3.87	4.02

The effect of plant extracts on the percentage and severity of infection with pathogenic fungi that infect the white fungus *Agaricus bisporus*

The extracts showed high inhibition efficacy for the pathogenic fungi, and they are very similar to the pesticide (Bavistin) that is recommended for controlling the fungal pathogens that infect *A. bisporus*. It was found that the infection percentage in the control treatment

(without the use of extracts or pesticide) was high, reaching 72.6% and 68.6% when the infection was caused by the pathogens *C. dendroides* and *T. aggressivum*, respectively (Table 7 and Figure 5). While garlic extract at a concentration of 1% recorded high efficacy in inhibiting both pathogenic fungi, *C. dendroides* and *T. aggressiveum*, reaching 0.0%, where it was superior to the pesticide, it is also noted that the infection percentage decreases for both fungi when the concentration of the plant extract is increased. The severity of infection decreased directly with the increase in the concentrations of the garlic and neem extracts, while the control treatment with the presence of the pathogens recorded high rates of infection severity, reaching 65.0% and 42.0% for the two pathogens, *C. dendroides* and *T. aggressivum*, respectively. No significant differences were shown for all the treatments compared to the pesticide bavistin. Through the results, it appears that using plant extracts results in a high inhibition of the pathogens when applied during the cultivation of white fungus in the mushroom development halls, unlike its laboratory application to some concentrations. Perhaps the reason is the interaction of several other factors in addition to the plant extracts that are applied for protection purposes, such as adding calcium carbonate to the covering soil, which prevents the growth of pathogens due to the change in pH to an alkaline state, but it does not have a strong effect when added alone, as was shown in the control treatment when adding the pathogenic fungus without treatment with plant extracts. In addition, the white fungus grows excellently on the compost medium, which encourages its growth compared to its growth on the industrial medium.

Table (7): Evaluation of the efficiency of plant extracts garlic and neem in reducing the percentage and severity of infection by the pathogenic fungi *C. dendroides* and *T. aggressivum* on the fruits of the white fungus *A. bisporus* in the production hall

Treatment	Concentration	Fungi			
		C. dendroides		*T. aggressivum*	
		infection %	severity of infection %	infection %	severity of infection %
Control	without pathogenic fungus	0.0	0.0	0.0	0.0
	with pathogenic fungus	72.66	65.06	68.60	42.00
Garlic	0.25	2.10	1.30	2.30	1.46
	0.5	1.83	1.10	1.93	1.36
	1	0.00	0.00	0.00	0.00
Neem	0.25	2.50	1.70	3.75	1.83
	0.5	2.13	1.50	2.93	1.53
	1	2.00	1.46	1.70	1.40
Bavistin	Recommended	1.56	1.70	0.96	1.53
LSD 5%		1.782	0.966	2.282	2.454

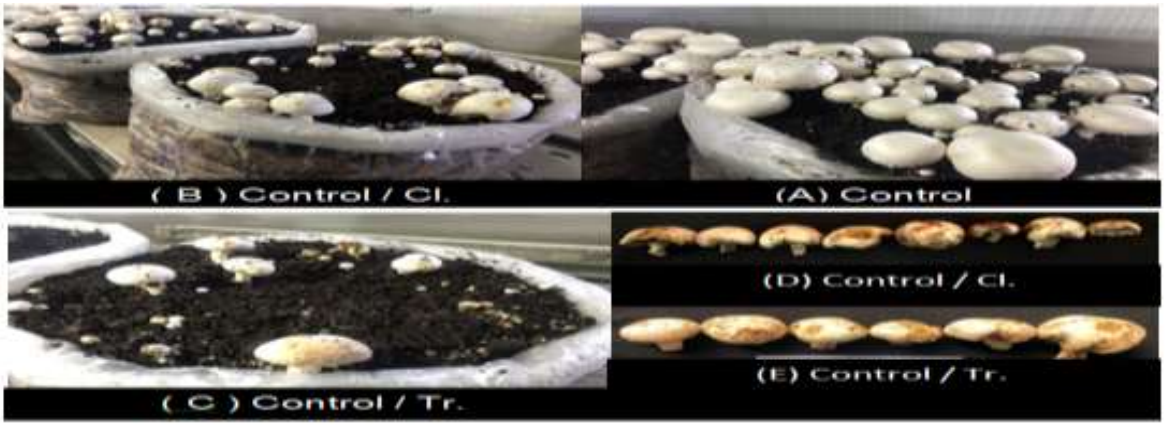

Figure (5): Percentage and severity of infection with the fruiting bodies of the white fungus *A. bisporus* in the production hall
A = control treatment without pathogenic fungus, B = control treatment for fungus *C. dendroides* alone, C =control treatment for fungus *T. aggressivum* alone, D = infected fruits in the control treatment for fungus *C. dendroides* alone, and E = infected fruits in the control treatment for fungus *T. aggressivum* alone.

The effect of plant extracts on the productive characteristics of white fungus that are infected with the two pathogenic fungi

The infection with the two pathogenic fungi led to an increase in the required period for the emergence of fruits (Table 8), as the white fungus in the control treatment (the pathogenic fungus without treatment) required 22.3 and 22.0 days for the two pathogenic fungi, *C. dendroides* and *T. aggressivum*, respectively, while the treatments with two concentrations of 0.25 and 0.5% for the two extracts showed good efficacy in the early trait of the emergence of fruiting bodies and limited the effect of the pathogenic fungi, as they did not record a significant difference from the control treatment (without pathogen), in which the fungus required 14.33 and 14.67 days for the emergence of fruiting bodies. The concentration of 1% of garlic and neem extract required a relatively longer period for the appearance of the fruiting bodies of the white fungus, and it was 15.67 and 15.33 days when treating the two pathogenic fungi, *C. dendroides* and *T. aggressivum*, respectively. It also appears that the amount of white fungus production was affected by the infection, as it significantly decreased when compared to the control treatment (without a pathogen), which amounted to 1116.3 g / bag. However, it also appears that the use of garlic and neem extracts led to a significant increase in productivity when compared to the control treatment (with the pathogenic fungus without treatment), where the production rate was 304.0 and 297.0 g/bag when infected with the two pathogenic fungi, *C. dendroides* and *T. aggressivum*, respectively. The use of a concentration of 0.5% of garlic extract showed a significant increase in the amount of production, reaching 1000.0 and 1049.0 g/bag when infected with the above two pathogenic fungi, respectively. On the other hand, it also appeared that all the concentrations treatments using garlic and neem extracts were relatively superior in terms of production compared to the treatment using the pesticide bavistin, which recorded the production amounts of 775.3 and 832.3 g/bag for the two pathogenic fungi above, respectively. It is noted that the required period for the emergence of fruiting bodies and the quantity of fruiting bodies are in direct correlation. The infection with pathogenic fungi affected the increase in the required period for the emergence of fruiting bodies, and this affects the amount of production. Perhaps the reason is attributed to the

increase in the growth period compared to the period of fruiting bodies production, which leads to the consumption of the nutrients in the compost growth medium, which leads to a lack of sufficient materials for the development of the fruiting bodies, and the increase in the period is due to the spread of the pathogen in the covered soil, which prevents its penetration by the fungal mycelium of the trophic fungus, whereas when using the extracts, it gave a greater chance for the fungal mycelium to penetrate the covered soil and start fruiting by suppressing the pathogens.

Table (8): Evaluation of the inhibitory efficiency of garlic and neem extracts against the pathogenic fungi *C. dendroides* and *T. aggressivum* on the productivity of the white fungus *A. bisporus* in the production hall

Treatment	Concentration	Fungi			
		C. dendroides		*T. aggressivum*	
		Period of appearing the fruit bodies /day	Weight of fruits /g	Period of appearing the fruit bodies /day	Weight of fruits /g
Control	without pathogenic fungus	14.33	1116.3	14.67	1099.7
	with pathogenic fungus	22.33	304.0	22.00	297.0
Bavistin	2.5 m/liter	15.33	775.3	14.33	832.3
Garlic	0.25	14.00	850.3	13.00	1049.0
	0.5	14.67	1000.0	14.33	939.0
	1	15.67	850.6	15.33	948.0
Neem	0.25	14.33	834.6	13.67	1355.0
	0.5	14.67	766.0	14.33	785.6
	1	15.00	807.0	14.67	828.6
LSD 5%		0.86	153.7	1.1	193.10

CONCLUSION

The fungus *T. aggressivum* is the first recorded in Iraq and was isolated from the medium and fruits of the white fungus *A. bisporus*. The isolates of the two pathogenic fungi, *Cladobotryum* spp. and *Trichoderma* spp., which were isolated from different culture media of the white fungus *A. bisporus*, had a relatively higher pathogenicity at a temperature of 25 C^{o}. The superiority of the alcoholic garlic extract in inhibiting the two pathogenic fungi, *C. dendroides* and *T. aggressivum* at a concentration of 1% on the neem oil extract in the laboratory and in the development hall. The plant extracts showed a low rate of inhibition against the white fungus *A. bisporus*. All treatments showed effectiveness in reducing the percentage and severity of infection and improving growth and productivity standards under hall conditions. It can be recommended to evaluate the efficiency of garlic and neem extracts in controlling other pathogens that infect the white fungus, *A. bisporus*, and research on other plant extracts to control the fungi, *C. dendroides* and *T. aggressivum*, which are safe for the white fungus**.**

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