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Isolating and Identifying Some Types of Bacteria and Fungi from wheat grains and studying the effect of some types of antibiotics and the aqueous and alcoholic extract of the *Alium sativum* plant on them

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***Abstract*— The study included the effect of Alium sativum aqueous and alcoholic *extracts* on the growth of bacteria and fungi isolated from the study The following types of bacteria appeared:Pseudomouas aeruginosa ,Aeromonas salmonicida ,Escherichia coli Using the diffusion method The aqueous extract was shown to be somewhat higher than the alcoholic extract in inhibiting the above-mentioned bacteria, In the past three decades, disease-resistant fungi and bacteria have emerged it caused major health problems all over the world through the pharmaceutical industry produced quantities of antibiotics. Unfortunately, resistance to antibiotics is increasingly important. Find plants with antifungal and antibacterial activity it has gained increasing importance in recent years due to the development of resistance. On the other hand, it is known that free radicals play an essential role in many diseases. Biochemical damage caused by free radicals to cells and tissues. If the average inhibition diameters at a concentration of 100% for the aqueous extract were (20.16.18) mm, respectively While the rates of inhibition diameters for the alcoholic extract reached (17,14,16) mm, respectively. As for the fungi isolated from the study, they are: Aspergillus .niger ,Rhizopus stolonifer . : Aspergillus candidus .Aspergillus fumigatus. Aspergillus flavus . Aspergillus ochraceus It was found that the aqueous and alcoholic extracts have an inhibitory effect on the growth of the isolated fungi when using the method of diffusion with the medium The results showed that the rates of inhibition diameters in the aqueous extract at a concentration of 100 mg/cm (6.5-0.3(cm. While the rates of inhibition diameters in the alcoholic extract were (7.0-1.9) cm . As for the antibiotics used in this study against the isolated bacteria, The bacteria isolated from the study showed resistance to (chloramphenicol, Piperacilline .,Cefotaxime). While the bacteria showed sensitivity to Amikacin and Gentamicin The statistical methods showed the superiority of Amikacin and Gentamicin to the rest of the antibiotics used in the study. The results also showed that all the fungi isolated from the study were resistant to nystatin They were sensitive to fluconazole, except for *A. niger*.**

Keywords— *Allium sativum*, wheat grains, aqueous extract, alcoholic extract, antibiotics

I. INTRODUCTION

Triticum aestivum is a herbal plant that contains 12-17% proteins and 1.5-1.2% fats, so it provides the adult person with more protein than he needs [1] . The presence of mycotoxins in foods and grains is one of the most serious underlying and affecting health and economic risks. The aflatoxin toxins are the most famous among the mycotoxins. Aflatoxins are toxic metabolites produced by fungi that pose a danger to humans and animals when contaminated grain products are used as food [2] [3]. So, the genus Aspergillus includes 200 species that grow on different materials and these various types of mushrooms are common in the soil in the form of restored organisms that have the ability to produce large numbers of conidia.

Aspergillus flavus is characterized by its ability to live on a number of food and organic sources, as well as live on grains. It causes harm to humans and animals. On the other hand, *Aspergillus fumigatus* is characterized by fast-growing green, mysterious colonies. The conidia are in the form of columns. This fungus is a pathogen to humans, as it affects the respiratory system and is also found in soil and seed [4] . As for *Aspergillus Candidus*. Thread grows on plant materials and causes respiratory damage to workers in grain mills if dust is released into the lungs, including spores, causing allergic diseases [5]. As for *Rhizopus stolonifer, it* causes contamination of food and animal feed through the production of mycotoxins and other biologically active compounds [6]. As *for Aeromonas salmonicida*, it was isolated from plant and animal foods such as fish, meat, milk and its products. Therefore, it is the source of human infection with this bacteria. This bacteria has resistance to many antibiotics, which poses a threat to humans and animals [7] .

Allium: According to Ref. [8] "the difficulty in determining the ancestor of garlic is due to the sterility of its varieties,"

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however, it is believed to be descended from a species called "*Allium longicuspis*," which grows wild in Central and Southeast Asia. The species called *Allium sativum* grows wild in areas where it has become a species (it is not native). Wild, vine, and British field garlic are all members of the *Allium ursinum*, *Allium vineale*, and *Allium oleraceum* families, respectively. In North America, wild garlic (*Allium vineale*) and wild onion (*Allium canadense*) commonly grow as weeds in fields. One type of garlic is known as elephant garlic (*Allium ampeloprasum*). Not real garlic. Single clove garlic originates in the Chinese province: Greece.[9].

II. MATERIALS AND METHODS

A. *Collect plant samples*

Wheat grains and Allium were collected from Nasiriyah markets .During the period from October to December 2022 . Three samples were randomly selected, at a rate of 1 mg for each sample of grains. They were preserved in nylon bags and brought to the laboratory for the purpose of studying them

B. *Isolation and identification fungi*

The fungi accompanying wheat grains were isolated using the Agar plate method on Potato Dextrose Agar medium, and incubated at a temperature of 25 °C for 7 days, after which the fungal colonies were examined under a light microscope depending on the phenotypic characteristics of the fungi. fungal species and genera, according to the taxonomic keys for the diagnosis of fungi [10].

C. *Isolation and identification bacteria:*

Bacteria in this study were isolated on a Nutrient Agar medium with three replicates and then incubated at 37°C for 24 hours. The bacteria were diagnosed using gram staining and biochemical tests, and strips were used.[11]

D. *Antibiotics Susceptibility Test :*

The sensitivity of bacterial isolates to antibiotics (Chloramphenicol, Aimkacin , Gentamicin , Piperacillin and Cefotaxime was tested by the disc diffusion method [12].

E. *Antifungal Susceptibility Test:* As mentioned in [13].

F. *Preparation of plant Extracts*

1) Aqueous garlic extract:

It was taken approx10 g of garlic cloves and added to it 100 ml of distilled water and mixed it with the mixer and then left the mixture for 30 minutes before filtering. The mixture was filtered using a Buechner funnel and using a special filter paper millipore with a diameter of 0.45 micrometer permeability to get rid of bacterial contaminants [14].

2) alcoholic extract:

Take 15 weights of garlic powder and add to it 200 ML ethyl alcohol at a concentration of 80%. Leave the mixture for 7 hours at a temperature of 60°C. Then filter it using filter paper. Then the filter is subjected to evaporation using a vacuum rotary evaporator until a thick liquid is obtained. Then the remaining liquid is dried using an oven at a temperature of 45° C For Dry Powder Of Alcoholic Garlic Extract [14]

III. RESULTS AND DISCUSSION

Isolation and identification of microorganisms in this study ,The diagnosis of fungi isolated from wheat grains was made: *Aspergillus niger*, *Rhizopus stolonifer*. *Aspergillus candidus*. *Aspergillus fumigatus*. *Aspergillus flavus*. *Aspergillus ochraceus* This result agrees with Ref. [15], They isolated several types of fungi from wheat and corn grains, such as *Aspergillus fumigatus*, *Aspergillus niger*. This may be attributed to the fact that the fungus has the ability to grow in low moisture content and tolerant drought conditions and low temperatures, which are considered as suitable factors for the growth of fungi [16]. On the other hand, the diagnosis of bacteria in this study was based on phenotypic, microscopic and biochemical examinations It includes a test strip. The bacteria were first diagnosed based on the phenotypic characteristics of the colonies by way of shape, color and smell, as well as the ability of the bacteria to ferment the lactose sugar present on the medium of Maconkey agar. Whereas, pink colonies of *E. coli* appeared on the medium of Maconkey agar due to its fermentation of lactose sugar small and dry with regular ends as well as being negative for the oxidase and urease assay and Vogues proskauer test Positive for red methyl and indole test [17]. As for the bacterium *Pseudomonas aeruginosa*, its hemolytic pale-coloured colonies appeared on the medium of the blood agar, and it was greenish-black in color due to its production of pyocyanin pigment and an odor similar to the smell of fermented grapes, as well as being positive for the examination of catalase and oxidase. [18]. As for *Aeromonas salmonicida*, it was positive for catalase and oxidase, methyl red test, negative for urease and indole, and Vogues proskauer test [19] [20]. The presence of this bacteria on the grain is due to its contamination with bacteria during the ripening stage, harvesting, or during the storage period as shown in this study.

Table (1) of results of biochemical tests for bacteria isolated in this study

Test	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aeromonas salmonicida*
Indole	+	-	-
Methyl red	+	-	+
Simmons citrate	-	+	+
Urease	-	-	-
Oxidase	-	+	+
Catalase	+	+	+
Vogues-proskauer	-	-	-

Table (2) The effect of different concentrations of *Allium sativum* aqueous extracts on the growth of the fungi isolated in this study:

Effect rates of aqueous extract	Colony diameters in the presence of aqueous extract				Average colony diameter (cm)	Inhibition percentage				isolated fungi
						Extract Concentration (mg/cm)				
	100	75	50	25		100	75	50	25	
1.725	1.0	1.6	2.1	2.2	8.5	88.2	81.1	75.2	74.1	*A. candidus*
3.125	1.6	3.3	3.6	4	8.5	81.1	61.1	57.6	52.9	*A. ochraceus*
4.0	2.3	2.7	5.2	6.1	8.5	72.9	68.2	38.8	28.2	*A. niger*
1.75	0.6	1.0	2.5	2.9	8.5	92.9	88.2	70.5	65.8	*A.fumigatus*
1.075	0.3	0.6	1.4	2	8.5	96.4	92.9	83.5	76.4	*A.flavus*
8	6.5	8.5	8.5	8.5	8.5	23.5*	0	0	0	*R .stolonifer*

All numbers mentioned in the above table are average of three replicate

Table (3) The effect of different concentrations of alcoholic extracts of *Allium sativum* on the growth of the fungi isolated in this study.

Effect rates of alcoholic extract	Average colony diameter in the presence of alcoholic extract				Average colony diameter (cm)	Inhibition percentage				isolated fungi
						Extract Concentration (mg/cm)				
	100	75	50	25		100	75	50	25	
2.2	1.9	2.2	2.2	2.6	8.5	77.6	74.1	74.1	69.4	*A.candidus*
3.47	2.8	3.2	3.7	4.2	8.5	67.0	62.3	56.4	50.5	*A.ochraceus*
5.97	2.7	5.6	7.6	8	8.5	68.2	34.1	10.5	5.8	*A.niger*
2.4	2.1	2.3	2.5	2.8	8.5	75.2	72.9	.70.5	67.0	*A.fumigatus*
2.0	1.9	1.9	2.0	2.4	8.5	77.6	77.6	76.4	71.7	*A.flavus*
8.1	7.0	85	8.5	8.5	8.5	17.6*	0	0	0	*R .stolonifer*

All numbers mentioned in the above table are average of three replicate

Table (2) (3) shows the effect of aqueous and alcoholic extracts of *Allium sativum* on the growth of the tested fungi. The rate of colony diameters was inversely proportional to the concentrations of the extract, as the rates of growth diameters decreased as the concentration of the extract increased. The average diameter of the fungal colonies treated with aqueous extract at a concentration of 100 mg/cm was between (6.5-0.3) mm and inhibition rates was (23.5-96.6%), while the diameters of colonies treated with alcoholic garlic extract at a concentration of 100 mg/cm were between (6.5-1.3) mm and in percentages of Inhibition (77.6-67.0)% The results of the effect of the aqueous and alcoholic extracts on the growth of the isolated fungi showed an inhibitory effect. It also appears from the results that the superiority of the aqueous extract over the alcoholic one is due to the removal of the allicin compound from the reaction by the organic solvent, and this reduces the activity against microbes [21]. The inhibitory activity of the allicin compound on the fungi depends on the inhibition of enzymes containing the thiol group (HS) through the oxidation of the thiol group present in the basic proteins [22]. The result that we obtained in this study is similar to what was found [23]. when they studied the effect of garlic extract on pathogenic fungi of wheat grains, where they found that allicin in garlic juice inhibited the radial growth of fungi. This study also agreed with [24]. who stated that allicin possesses a high inhibitory activity in fresh garlic extract.

Table (4) The effect of different concentrations of aqueous extracts of Allium sativum on the bacteria isolated in this study

Inhibition diameters of the aqueous extract	Diameters of damper zones (mm) aqueous extract				Type of bacteria
	100 %	75%	50%	25%	
14	20	15	12	9*	*E. coli*
11.25	16	13	9	7	*P.aeruginosa*
13.5	18	16	11	9	*A. salmonicida*

Table (5) The effect of different concentrations of alcoholic extracts Allium sativum on the bacteria isolated in this study

Inhibition diameters of the alcoholic extract	Diameters of damper zones (mm alcoholic extract				Type of bacteria
	100%	75%	50%	25%	
11.5	17	13	9	7	*E. coli*
9	14	10	7	5	*P.aeruginosa*
10.75	16	12	8	7	*A. salmonicida*

Table (4) (5) shows that the most effective concentration on the isolated bacteria is 100 mg / cm, if the inhibition rate of the aqueous extract of *E. coli* is 20 mm, *P. aeruginosa* 16 mm and *A. salmonicida*, 18 mm So, the inhibition rate of alcoholic extract of *E. coli* was 17 mm mm, *P. aeruginosa* 14 mm and *A. salmonicida*

bacteria., 16 mm. The results showed that the effect of the aqueous and alcoholic extracts on the growth of the tested bacteria had a significant inhibitory effect under the level of 0.05. The results showed that the aqueous extract gave a somewhat higher inhibition than the alcoholic extract This result is in agreement with [25]. He mentioned that the aqueous garlic extract is stronger than the organic extracts against *Staphylococcus aureus and P. aeruginosa E. coli* .The effectiveness of garlic is due to the presence of the compound allicin, which can be obtained from crushing garlic bulbs, which leads to the release of the enzyme Allicinase, which converts the amino acid Alliin to Allicin, which has antimicrobial activity [14].

Table (6) Antifungal Susceptibility:

antifungals				isolated fungi
Nystatin		Fluconazole		
Sensitivity %	Retarding diameters (mm)	sensitivity %	Retarding diameters (mm)	
0	0(R)	100	42(S)	*A.candidus*
0	0(R)	60	9.0(I)	*A.ochraceus*
0	0(R)	0	0(R)	*A.niger*
0	0(R)	100	46(S)	*A.fumigatus*
0	0(R)	100	45(S)	*A.flavus*
0	0(R)	100	37(S)	*R .stolonifer*

(R) Resistive (>9), (I) Intermediate (9-15) ,(S)Sensitive (<15)

The drug sensitivity of the antifungal Nystatin and Fluconazole was tested against the fungi isolated in this study as shown in Table (5). The results showed that the isolated fungi were sensitive to the antifungal Fluconazole, except for A. niger, which showed resistance to this antifungal. As for the anti-Nystatin, all fungi showed resistance to this antibiotic. These results are in agreement with Ref. [26]. where he stated that the azole group, including the anti-Fluconazole, is characterized by its ability against fungi. The reason for the effectiveness of azole against fungi is due to the effect on the cytochrome enzymes involved in the manufacture of Ergosterol, as it inhibits the synthesis of the Ergosterol complex in the plasma membrane of the fungal cell [27].

Table (7): Drug sensitivity test for antibiotics against bacteria isolated in this study

isolated bacteria						Ant-ibiotics
A. salmonicida		*P. aeruginosa*		*E.coli*		
sensitivity %	Retarding diameters (mm)	sensitivity %	Retarding diameters (mm)	sensitivity %	Retarding diameters (mm)	
0	0(R)	30	7.5(R)	28	7(R)	Chloramphenicol
97	16.5(R)	100	20(S)	100	20(R)	Aimkacin
100	25(S)	64.2	9.0(I)	100	22(S)	Gentamicin
0	0(R)	0	0(R)	0	0(R)	Piperacillin
0	0(R)	0	0(R)	33.3	5(R)	Cefotaxime
1.7		1.75		2.7		L .S.D (P<0.05)

The results showed that the isolated bacteria showed resistance to antibiotics, except for two antibiotics, Amikacin and Gentamicin This is attributed to the fact that these antibiotics belong to the aminoglycoside group, and that their mechanism of action is by inhibiting protein synthesis inside the cell, due to their ability to bind to the small unit of the 30s ribosome, allowing the entry of wrong sequences of amino acids in the peptide chains, leading to a wrong reading of the mRNA code, and then producing inactive proteins [26]. On the other hand, the emergence of bacterial resistance to anti-penicillins and anti-cephalosporins, where *E.coli* bacteria are a good producer of many beta-lactam enzymes and their ability to produce beta-lactamase enzymes that encode for their production chromosomally loaded genes [28]. In addition to enzymatic resistance, there are other methods, including the methods of flow systems, which is the system of pumping the drug out. The occurrence of genetic mutations in the genes encoded for these systems raises the resistance [28].

IV. Conclusion & Recommendations

Conclusion, the study showed the inhibitory effects of Alium sativum plant extracts on both bacteria and fungi isolated from the study, as well as the resistance and sensitivity of the isolated bacteria and fungi to various antibiotics and antifungals. The inhibitory power of the garlic plant was higher than the other plant extract and antibiotics which used in this study.

Overall, the research highlighted the potential of Alium sativum extracts as antimicrobial agents and provided valuable insights into the antibiotic resistance patterns of the isolated bacteria and fungi. These findings are significant in the context of addressing the global challenge of antimicrobial resistance and the search for alternative antimicrobial agents.

The trend towards plant extracts as a medicinal alternative due to the many challenges that society currently faces as a result of increasing resistance to antibiotics. Reducing the use of antibiotics to reduce the rate of resistance by bacteria and fungi.

Conflict of Interest

Authors declare that they have no conflict of interest.

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