

Article

# Isolation and Identification of Toxin-Producing Fungi Associated with Some Potato Chips and Inhibition of Their Growth Using Plant Extracts

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**Abstract:** This study involved the isolation and identification of fungi contaminating various types of potato and corn chips. 91 fungal samples were isolated, belonging to multiple genera, with a clear dominance of the genus *Aspergillus*, along with species such as *A. niger*, *A. flavus*, *A. terreus*, *A. oryzae*, and *A. parasiticus*, as well as *Penicillium* and other species such as *Alternaria* spp. and yeasts. The results confirmed that all isolates produced aflatoxins. Evaluations were conducted to test the effectiveness of alcoholic plant extracts from onion, clove, cinnamon, and rosemary in inhibiting the growth of these fungi. The results showed a variation in the degree of inhibition, depending on the fungal species and extract concentration. The variation in fungal response is attributed to the diversity of cell wall composition and fungal metabolic pathways, as these characteristics influence the ability of fungi to resist or be affected by active plant compounds. The study indicated the vital role of natural chemical compounds in inhibiting fungal growth through mechanisms such as disruption of cell membranes, inhibition of vital enzymes, and exposure to oxidative stress. These findings highlight the potential of using plant extracts as natural flavorings for gypsum chips or as safe and effective antifungals as alternatives to chemical pesticides. Their application is important as part of food safety strategies and to reduce food contamination with mycotoxins.

**Keywords:** *Aspergillus*, Aflatoxin, Ammonia Detection.

## Introduction

According to World Health Organization statistics, one in 10 people becomes ill as a result of consuming contaminated food, which leads to the death of 420,000 people annually. Therefore, food safety from microscopic contaminants is considered one of the main economic factors and a priority for the Public Health Organization [1]. Fungi grow widely in various types of foods such as (corn, potatoes, grains, milk and its derivatives, meat, nuts, fruits, legumes and coffee), and fungal contamination occurs at any stage of production after harvest or storage and under a wide range of environmental conditions [2], [3]. Fungi grow widely in various types of foods such as (corn, potatoes, grains, milk and its derivatives, meat, nuts, fruits, legumes and coffee), and fungal contamination occurs at any stage of production after harvest or storage and under a wide range of environmental conditions [4], [5]. The majority of fungi are medium-sized fungi, varying in temperatures at which they grow, ranging from 5-35 degrees Celsius, with ideal growth temperatures between 25 and 30 degrees Celsius.

However, fungi exist and grow well outside the average temperature range; Some are cold tolerant and able to grow near or below 0°C, while others are heat tolerant and grow above 40°C [6].

One of the most important problems related to food safety is the presence of natural contaminants such as mycotoxins [7], [8]. Contamination of various food materials with mycotoxins is a major problem facing societies in tropical and subtropical regions. Where climatic conditions and agricultural storage practices are conducive to the growth of fungi and the production of toxins [9], [10], [11], [12], food becomes contaminated. That humans consume a large number of fungi that secrete many toxins, such as aflatoxin, which leads to harmful effects on human health as a result of the consumption of mycotoxins, and the contamination of agricultural products with mycotoxins still occurs in the countries of the developed world despite modern agricultural applications. The existence of a system has led to Food processing and organized marketing aim to reduce the level of mycotoxin contamination. In general, mycotoxin contamination occurs in foods traded in the markets as a result of poor storage conditions [13]. Among the fungal species capable of producing a wide range of mycotoxins are *Alternaria* and *Fusarium* [14], [15].

In 2019, mycotoxins were reported to be the main risk in food products at the EU border, more recently in 2020, a survey of global food crop contamination reported a 25% incidence of mycotoxins Above EU and Codex limits [16]. It is certain that mycotoxin production may change under various fungal growth conditions such as physiological environmental factors such as temperature, water activity, pH, oxygen concentration, and biotic factors including microbial activity [17]. Mycotoxins are toxic chemical compounds that are secondary metabolites produced by fungi or molds. These mycotoxins found in food have a significant impact on human health. Mycotoxins are associated with diseased or rotting crops. Although visible mold contamination can be superficial, the effects of some foodborne mycotoxins are severe, and symptoms of severe disease appear. Very quickly. There are five types of mycotoxins or groups of mycotoxins found in food: deoxynivalenol/nivalenol, zearalenone, ochratoxin, fumonisins and aflatoxin [18], [19].

Toxic fungi contaminating agricultural grains are traditionally divided into two groups. The first type is fungi that invade seed crops and are described as “field” fungi, for example, *Cladosporium*, *Fusarium*, *Alternaria* spp. , which are said to gain access to seeds during plant development, and the second type are storage fungi such as *Aspergillus* and *Penicillium* spp. , which multiply during storage [20]. This division is not currently considered precise because according to J.D. Miller [21] four types of toxic fungi can be distinguished: (1) plant pathogens such as *Fusarium graminearum* and *Alternaria alternata*; (2) Fungi that grow and produce mycotoxins on senescent or stressed plants, such as *F. moniliforme* and *Aspergillus flavus*; (3) Fungi that initially colonize the plant and increase susceptibility to post-harvest contamination such as, *A. flavus*; (4) Fungi in soil or decaying plant matter that occur on grains growing in the field and later multiply in storage if conditions permit such as *P. verrucosum* and *A. ochraceus*

Mycotoxins have significant effects on human health, as they cause kidney poisoning, immunosuppression, birth defects, and types of cancer. These toxins are capable of causing acute and chronic effects in humans and animals, ranging from death or disruption of the central nervous system, heart, blood vessels, and lungs [22], [23]. In Iraq, a number of fungi were isolated for a group of foods, such as meat, chicken, tomatoes, potatoes, cucumbers, onions, and bread. Among the types of fungi isolated were *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Brettanomyces* sp [24]. Many medicinal plants have antifungal activity due to their active compounds such as phenols, volatile oils, and glycosides. Research is particularly focused on plants rich in flavan-3-ols and anthocyanins, as these exhibit antioxidant activity and are believed to be more effective against resistant pathogens than synthetic drugs, with fewer side effects [25], [26].

Examples of plants and their active compounds include:

Rosemary: Contains carnosic acid and rosmarinic acid, which have antifungal and anti-inflammatory properties, as well as antioxidant and immune-boosting effects.

[27].

Cinnamon: Contains compounds such as cinnamon and essential oils with antifungal and antioxidant effects [28], [29].

Onions: Contain sulfur compounds such as allicin, propene sulfide, and thiosulfate, which disrupt fungal cell function. They also contain the antioxidant S-allylcysteine and quercetin, which inhibit energy production and fungal cell wall formation. They also contain phenolic acids, gallic and caffeic acid, which reduce fungal reproduction and activity [30], [31].

These compounds disrupt fungal plasma membranes and cause the loss of vital elements, leading to their death. This makes them promising options for combating fungal infections with fewer side effects compared to synthetic drugs. For the sake of food safety and the use of plant extracts that are safe for human consumption instead of manufactured preservatives, and given the importance of the subject and its relationship to human health and the nature of human food, we decided to conduct a study aimed at isolating and identifying the fungi contaminating some types of potato and corn chips and their ability to produce aflatoxin and studying the inhibitory effects of alcoholic extracts of some plants.

## Materials and Methods

### 1. Sample collection:

20 samples of potato and corn chips were collected randomly from local markets in the Governorate, three samples for each type, for the purpose of obtaining isolates of fungi that produce aflatoxins.

### 2. Agricultural media:

A- medium of potato dextrose agar extract (PDA Potato dextrose agar).

This medium was prepared in the laboratory according to the manufacturer's instructions by dissolving 39 grams of the medium in 1 liter of distilled water, then sterilizing it in an autoclave at a temperature of 121°C and at a pressure of 1 atmosphere for 20 minutes, then cooling it to 45°C, after which the antibiotic chloramphenicol was added to it at a concentration of 250 mg/l. The medium was used for the purpose of growth. Isolation and diagnosis of fungi.

#### B. Medium Coconut Extract Agar (CEA)

Prepare this laboratory medium by mixing 300 ml of distilled water with 100 grams of shredded coconut, then heat the resulting mixture for 20 minutes, then filter it using several layers of sterile gauze. Add 1.5% of the agar to the filtrate and complete the volume to 300 ml of distilled water, then sterilize the medium. [32] The medium was used for the purpose of detecting aflatoxin-producing fungi.

#### C. Zabek medium Czapek Aga

This medium was prepared in the laboratory by dissolving 33.4 g of the ready-made powder in 1 liter of distilled water according to the manufacturer's instructions, and this medium was used to diagnose fungi [33].

### 3. Isolation and purification of fungi

The chips samples were cut into small pieces of 3 mm in size, then four pieces were taken from each sample and planted using a sterile needle in petri dishes with a diameter of 9 cm containing PDA culture medium, 3 cm from the edge of the dish, by placing four pieces of chips 3 cm from the edge of the dish, and a piece of A fifth in the middle of the dish and the process was repeated three times (repeats) for each material. All dishes were incubated at two temperatures at 27°C for a week. After the fungal growth appeared, the cultures were grown by taking a swab from the edge of the colony with a sterile needle, and they were grown again on the same medium and incubated for another seven days at the same temperature as before. Thus, pure cultures were obtained. The samples were kept in sterile test tubes containing PDA medium at an angle. slant discs were planted on the culture medium for seven days. I prepared these media for the purpose of preserving the fungi for a longer period and preserving them without contamination until the study was completed. They were kept in the refrigerator at a temperature of -4° C.

#### 4. Detection of aflatoxins using ammonia solution

The ability of fungal isolates to produce aflatoxin was detected using the coconut medium prepared in paragraph (B). It was poured into dishes with a diameter of 9 cm. Then three replicates were inoculated with disks of fungal isolates growing on PDA medium with a diameter of 5 mm, at one week old, in the center of the dish. The process was repeated on All the isolates studied were then incubated in the dishes at a temperature of 25°C for a week. The isolates capable of producing aflatoxin were detected using a 20% ammonia solution through the use of filter papers saturated with the solution in the cover of the dish. Then the dishes were incubated upside down for 7-10 days at two temperatures of 27 and 37. The ability of fungi to produce aflatoxins is inferred from the occurrence of a color change in fungal colonies from transparent to pink or red [34]. of the isolated fungi

#### 5. Morphological Identification

The fungal isolates obtained were identified based on the phenotypic characteristics of the colony and microscopic examination on PDA and CZA medium using an optical microscope and at several magnification powers based on the shape of the fungal hyphae and according to the approved taxonomic keys [35], [36].

The occurrence rate and frequency were calculated according to the following equations:  
Percentage of occurrence (%) =  $\frac{\text{number of samples in which one species appeared}}{\text{Number of total samples}} \times 100$

Frequency percentage (%) =  $\frac{\text{number of isolates of one genus}}{\text{Total number of isolates of all types}} \times 100$

#### 6. Preparation of alcoholic extracts (onion, clove, cinnamon, rosemary):

The plants were obtained from local markets in . The samples were thoroughly washed with distilled water to remove dust and impurities and dried by placing them in a tray in an oven at 50°C until the weight stabilized. The selected parts (leaves, bark, and fruit) were then ground using an electric grinder to obtain a dry powder. To obtain the alcoholic extract, 20 grams of plant powder was weighed for each plant and placed in a 250 ml beaker containing 200 ml of 70% alcohol. The beakers were sealed with cotton and covered with aluminum foil. The beakers were placed in a shaker for 24 hours, after which the mixture was filtered through several layers of gauze, and the resulting solution was filtered through filter paper. A centrifuge was then used to obtain a clear, impurity-free filtrate. The filtrate was placed in flat dishes and dried in an electric oven until completely dry. The extracts were collected by scraping and stored in opaque tubes in the refrigerator. Until us

#### 7. Preparation of Alcoholic Extract Concentrations:

A series of concentrations (0.5, 1, 2.5, 5, and 10) mg/ml were prepared, in addition to a control concentration without additions. The concentrations were prepared according to the law  $C_1 V_1 = C_2 V_2$ , as follows:

1. Dissolve 50 mg of the extract in a volume of water and add to 100 ml of culture medium to obtain a concentration of 0.5.
  2. Dissolve 100 mg of the extract in a volume of water and add to 100 ml of culture medium to obtain a concentration of 1 mg/ml.
  3. Dissolve 250 mg of the extract in a volume of water and add to 100 ml of culture medium to obtain a concentration of 2.5 mg/ml.
  4. Dissolve 500 mg of the extract in a volume of water and add to 100 ml of culture medium to obtain a concentration of 5 mg/ml.
  5. Dissolve 1 g of the extract in a volume of water and add to 100 ml of culture medium to obtain a concentration of 10 mg/ml.
- #### 8. Testing the effect of alcoholic extracts on the growth diameters of fungi isolated from potato gypsum samples.

PDA culture medium was used. The culture medium was prepared according to the manufacturer's instructions on the package. The antibiotic chlorphenicol was added to inhibit

bacterial growth. It was then autoclaved and cooled to 45°C. The plant extracts prepared in the previous paragraph were mixed at concentrations of 0.5, 1, 2.5, 5, and 10 mg/ml, in addition to the control medium without any additions. The medium was then poured into three replicates for each concentration and left to solidify. A disc of growing fungi was then placed in the center of the dish. The dishes were placed in an incubator at 28°C for 7 days. The diameter of the growing colony was then measured at the rate of two perpendicular diameters, and the results were recorded.

## Results

### Isolation of fungi from various types of chips

The results of isolating fungi from different types of chips (as shown in Table 1), which were taken randomly from the local markets of the Governorate, showed that all samples were accompanied by fungi, except for the Hala chips type and the Mino type. No fungal contamination was recorded.

**Table 1.** Number of fungal isolates for each type of chips.

	Name of the material	Origin	Number of fungal isolates
1	chips Hala	Iraq	0
2	Lays chips with salt and vinegar	Iraq	1
3	Lays chips with tomatoes	Iraq	2
4	Lays chips with herbs	Iraq	2
5	Lays chips with parpequ	Iraq	1
6	Lays chips with French chees	Iraq	4
7	Lays chips with hot pepper	Iraq	3
8	Lays chips with salt	Iraq	2
9	Lays chips with yogurt	Iraq	3
10	For your eyes chips	Iraq	30
11	mino chips	Iraq	0
12	Chips violet flowers	Iraq	6
13	Bouchar Panda	Iraq	3
14	Getos chips	Egypt	4
15	chips mazmaz	Iran	8
16	Chips Evening	Iraq	4
17	Chips Baz	Iraq	11
18	Indomie with vegetables	Iraq	3
19	Indomie with chicken	Iraq	2
20	Homemade popcorn	Iraq	3

Table 2) shows that the highest number of isolates was for fungal isolates, reaching 84 samples, at a rate of 92.31%, and that the lowest number of isolates was among yeast isolates, at 7 isolates, at a rate of 7.69%.

**Table 2.** Types of isolates from chip samples at a temperature of 27 and an incubation period of one week.

Type of isolates	the number	Percentage %
Fungi	84	92.31%
Yeasts	7	7.69%
the total	91	100%

Corn and potato crops are contaminated with associated fungi, either during the harvest period or during the storage period until they reach the food factories. This contamination occurs as a result of poor storage, such as humidity and inappropriate temperatures, which makes it an appropriate environment for the growth of fungi, or air pollution with fungal spores, thus transferring them to raw manufacturing materials, packaging materials, and workers [37], [38], [39], [40].

Table 3 shows that the highest number of isolates was of the fungus *Aspergillus niger* with 30 isolates and a percentage of 32.96%, followed by the fungus *Aspergillus flavus* with 20 isolates, and that fungal isolates were higher than yeasts, reaching 84 isolates, while yeasts reached 7 isolates out of a total of 91 isolates.

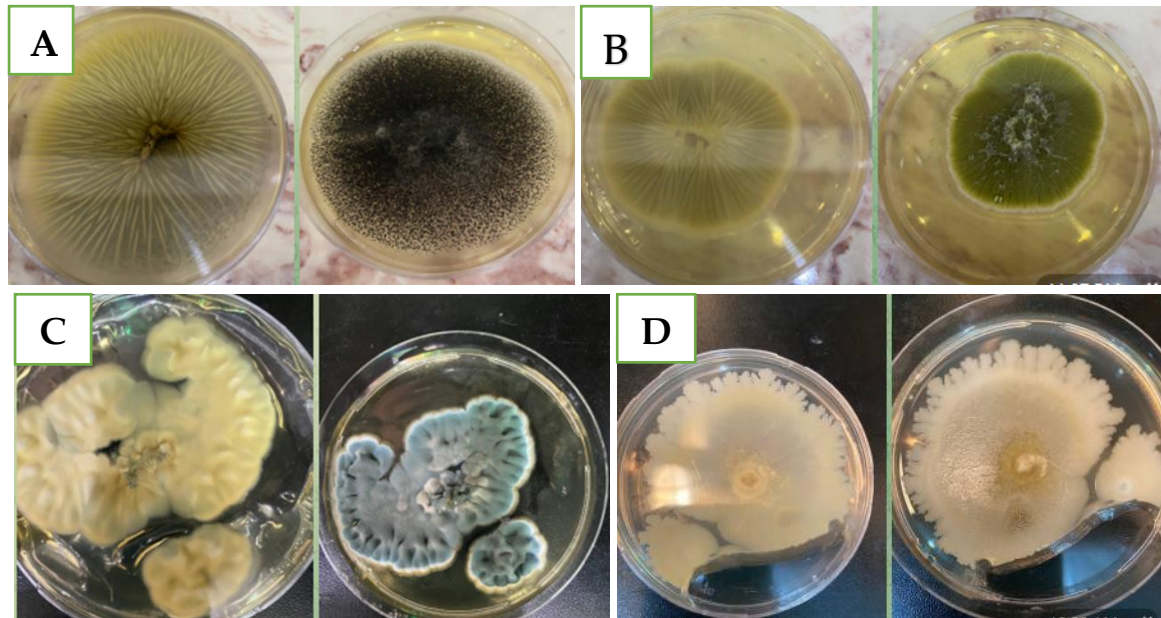
**Table 3.** Frequency percentages of fungi and yeasts isolated from chips samples at 27 degrees for a week.

	Isolated fungi	Number of isolates	Percentage %
1	<i>Aspergillus niger</i>	30	%32.96
2	<i>Aspergillus flavus</i>	20	%21.97
3	<i>Altrenaria spp</i>	1	%1.09
4	<i>Penicillium spp</i>	6	%6.59
5	<i>Aspergillus terreus</i>	7	%7.69
6	<i>Aspergillus oryzae</i>	6	%6.59
7	<i>Aspergillus prasiticus</i>	4	%4.39
8	<i>Aspergillus flavps</i>	10	%11.11
9	Yeasts	7	%7.69
	The total	91	100%

Fungi spread and grow in different environments because they can tolerate different ranges of temperatures and acidic environments, but they have preferred temperatures for their optimal growth, as they can grow in a range of 12-65 degrees Celsius, and the pH of the growth sites is between 2.1 and 8.8 [41]. The genus *Aspergillus* represents a diverse group of fungi that are among the most abundant in the world. Germination of spores can lead to the formation of mycelium. *Aspergillus* can reproduce sexually and asexually and to this end, it produces conidiophores and ascocarps that form conidia and ascospores, respectively, and is characterized by its ability to produce carcinogenic secondary compounds [42], [43].

They are found in various environments, including soil, seeds, roots, compost, spices, foods and various plants. They are considered destructive factors when it comes to agricultural products before and after harvest [44] It is often isolated from house dust, soil, dried nuts, fruits and seeds as well as various types of untreated textile materials such as hemp, jute and cotton. Hence, this type is

often found in abundance in the textile industry. *A. niger* can also contaminate meat and eggs, causing progressive spoilage; Sun-dried spices and fruits may also contain *A. niger* [45][46] In another study, 73 fungal isolates were isolated and identified from some fruits and foods in the markets, where the highest percentage was 21.9% for the *Penicillium* fungus and then 17.8% for the *Rhizopus* fungus. A number of fungi were producers of mycotoxins, such as: *Rhizopus*, *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Cladosporium*, *Botrytis*, *Geotrichum*, and *Colletotrichum* [47].



**Figure 1.** Shows some pictures of the front and back of some fungal colonies, isolated from some type of potato chips and corn, growing on PDA medium at 27°C for 7 days: *A-Aspergillus niger*, *B-Aspergillus flavus*, *C-Pincelium* *D-yeasts*.

**Percentage of impressions**

Table (4 ) shows the percentage of appearance of fungi, where the fungus *Alternaria* recorded the highest appearance rate of 100% in Popcorn Panda, and the fungus *Aspergillus flavus* recorded a percentage of appearance of 75% in a plaster sample for your eyes, and the fungus *Aspergillus nIger* recorded a percentage of appearance in all samples under study, and yeasts recorded a percentage Appearance reached 57.1% in Mazmaz chips , as Table (5) shows the number of fungal species in each sample of the study. The reason for the appearance of the *Aspergillus* fungus in foodstuffs is that the species of this genus have the ability to secrete a large number of enzymes that decompose food substances that are used for nutrition and growth, as well as increasing the capacity of its spread, especially since some of its species can grow in low moisture content, in addition to the relative density of the spores that Produce it

**Table 4.** Of the percentage of fungi appearing in samples.

Name of the material	orig in	<i>A.ni</i> <i>ge</i>	<i>A.flav</i> <i>us</i>	<i>Altrena</i> <i>ria spp</i>	<i>penicilli</i> <i>um</i>	<i>A. terre</i> <i>us</i>	<i>A.ory</i> <i>za</i>	<i>A. prasi</i> <i>ticus</i>	<i>A.flav</i> <i>us</i>	<i>Yea</i> <i>st</i>
1 chips Hala	Iraq	0	0	0	0	0	0	0	0	0
2 Lays chips with salt	Iraq	6.6					16.6			

	and vinegar									
3	Lays chips with tomatoe s	Iraq	.33				16.6			
4	Lays chips with herbs	Iraq	3.3				16.6			
5	Lays chips with parpequ	Iraq	3.3				16.6			
6	Lays chips with French chees	Iraq	3.3						10	
7	Lays chips with hot pepper	Iraq	3.3					25	10	
8	Lays chips with salt	Iraq	3.3				16.6		10	
9	Lays chips with yogurt	Iraq	3.3						10	
10	For your eyes' chips	Iraq	20	70		33.3	28.5	25	30	28.5
11	mino chips	Iraq	0	0	0	0	0	0	0	0
12	Chips violet flowers	Iraq	3.3	5		33.3	28.5	25		
13	Bouchar Panda	Iraq	13.3	5	100	16.6		16.6		
14	Getos chips	Egy pt	3.3				14.28		10	14.2
15	chips mazmaz	Iran	10				14.28	25	10	57.1
16	Chips Evening	Iraq	6.6	5			14.28			

17	Chips Baz	Iraq	3.3								
18	Indomie with vegetables	Iraq	3.3	5		16.6					
19	Indomie with chicken	Iraq	3.3								
20	Homemade popcorn	Iraq		5						10	

Table (5) shows the number of fungal species isolated from each gypsum sample. The most common fungi found in the samples were of the genus *Aspergillus*, particularly the following species: *Aspergillus niger* (30 isolates), *Aspergillus flavus* (20 isolates), Other species such as *A. terreus*, *A. oryzae*, *A. parasiticus*, and others were also present in smaller numbers. The genus *Penicillium* was represented by 6 isolates. Yeast isolates were relatively few, amounting to 7 isolates.

**Table 5.** Number of fungi in each sample.

	Name of the material	orig in	<i>A.ni</i> <i>ge</i>	<i>A.flav</i> <i>us</i>	<i>Altrena</i> <i>ria spp</i>	<i>penicilli</i> <i>um</i>	<i>A.</i> <i>terre</i> <i>us</i>	<i>A.ory</i> <i>za</i>	<i>A.</i> <i>prasi</i> <i>ticus</i>	<i>A fla</i> <i>vp</i>	<i>Yea</i> <i>st</i>
1	chips Hala	Iraq	0	0	0	0	0	0	0	0	0
2	Lays chips with salt and vinegar	Iraq	2	1				1			
3	Lays chips with tomatoe s	Iraq	1					1			
4	Lays chips with herbs	Iraq	1					1			
5	Lays chips with parpequ	Iraq	1					1			
6	Lays chips with French chees	Iraq	1							1	

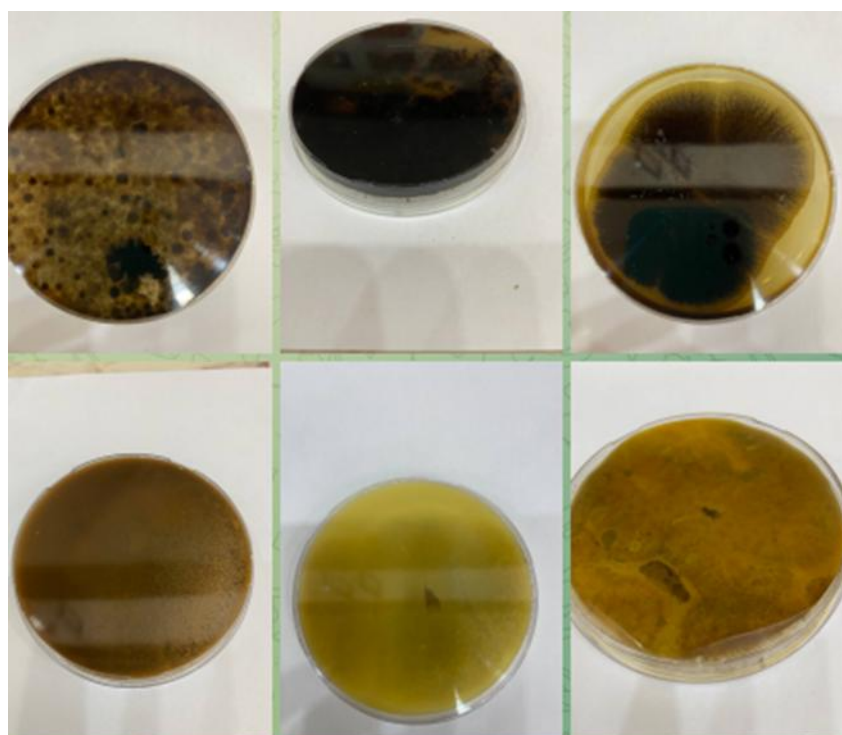
7	Lays chips with hot pepper	Iraq	1					1	1		
8	Lays chips with salt	Iraq	1				1		1		
9	Lays chips with yogurt	Iraq	1						1		
10	For your eyes' chips	Iraq	6	14		2	2		1	3	2
11	mino chips	Iraq	0	0	0	0	0	0	0	0	0
12	Chips violet flowers	Iraq	1	1		2	2		1		
13	Bouchar Panda	Iraq	1	1	1	1		1			
14	Getos chips	Egypt	4				1			1	1
15	chips mazmaz	Iran	3				1		1	1	1
16	Chips Evening	Iraq	2	1			1				
17	Chips Baz	Iraq	1								
18	Indomie with vegetables	Iraq	1	1		1					
19	Indomie with chicken	Iraq	1								
20	Homemade popcorn	Iraq	1	1						1	
	Total		30	20	1	6	7	6	4	10	7

### Detection of fungi producing aflatoxins

The detection results in table (6) showed that all fungi were able to secrete aflatoxin toxins, at a rate of 100%. This was inferred by the change in the color of the base of the colonies from transparent to dark color, as in the figure (2), and this was mentioned by Saito, M., & Machida, S. (1999)

**Table 6.** The percentage of fungi's ability to produce aflatoxins.

	Isolated fungi	Number of isolates	Percentage %
1	<i>Aspergillus niger</i>	30	%35.72
2	<i>Aspergillus flavus</i>	20	%23.87
3	<i>Altrenaria spp</i>	1	%1.20
4	<i>Penicillium spp</i>	6	%7.14
5	<i>Aspergillus terreus</i>	7	%8.33
6	<i>Aspergillus oryzae</i>	6	%7.14
7	<i>Aspergillus prasiticus</i>	4	%4.77
8	<i>Aspergillus flavps</i>	10	%11.90
	The total	84	100%



**Figure 2.** Shows the back side of some fungal colonies growing on coconut media at a temperature of 27°C for a period of 7 days to test the ability of the fungus to produce aflatoxin after adding ammonia solution.

**Table 7.** The effect of different concentrations of alcoholic extracts on the growth diameters (mm) of a number of isolated fungi.

Fungi	plant extracts	Con.					F*E
		0	1	2.5	5	10	
Niger	onion	77.83	69.67	56.50	44.17	27.83	55.20
	cloves	72.83	64.67	52.00	31.33	18.83	47.93

	cinamm	73.33	68.33	57.33	42.67	21.00	52.53
	rosmaty	77.00	69.33	54.33	40.67	77.00	63.67
Flavus	onion	69.00	61.33	53.00	42.33	28.00	50.73
	cloves	68.00	52.33	42.67	31.00	19.00	42.60
	cinamm	69.33	53.67	40.67	21.00	12.67	39.47
	rosmaty	67.67	61.00	55.33	42.00	34.67	52.13
Terrus	onion	76.00	62.67	54.33	32.00	19.67	48.93
	cloves	78.00	54.67	40.00	21.33	18.00	42.40
	cinamm	70.00	60.00	40.67	30.67	20.67	44.40
	rosmaty	75.00	68.00	53.33	42.33	28.00	53.33
Pencilum	onion	74.67	70.00	51.00	42.67	23.00	52.27
	cloves	76.33	61.33	41.00	33.33	17.67	45.93
	cinamm	76.33	64.00	50.67	41.00	21.67	50.73
	rosmaty	71.67	70.00	61.33	44.33	31.00	55.67
Yest	onion	56.67	41.00	33.00	28.00	21.33	36.00
	cloves	55.67	36.67	28.00	18.00	12.33	30.13
	cinamm	57.00	43.33	31.00	28.00	20.33	35.93
	rosmaty	52.00	43.00	38.00	26.33	20.67	36.00
L.S.D		F*E*C:3.893			F*E:1.741		
	Averg conce.	69.72	58.75	46.71	34.16	24.67	
L.S.D.	Con.:0.870						Averg E
E*C	onion	70.83	60.93	49.57	37.83	23.97	48.63
	cloves	70.17	53.93	40.73	27.00	17.17	41.80
	cinamm	69.20	57.87	44.07	32.67	19.27	44.61
	rosmaty	68.67	62.27	52.47	39.13	38.27	52.16
L.S.D.		E*C:1.741			E:0.779		
							Averg F
F*C	niger	75.25	68.00	55.04	39.71	36.17	54.83
	flavus	68.50	57.08	47.92	34.08	23.58	46.23
	terrus	74.75	61.33	47.08	31.58	21.58	47.27
	pencilum	74.75	66.33	51.00	40.33	23.33	51.15
	yest	55.33	41.00	32.50	25.08	18.67	34.52
L.S.D.		F*C:1.946			F:0.870		

The table(7) shows that yeasts were most affected by the concentrations of alcoholic extracts than the rest of the fungi, with significant differences. As for the effect of the type of alcoholic extracts, the clove extract was the most inhibitory, with an average diameter of 41.80 mm compared to the control treatment, while the rosemary extract was the least effective, giving an average diameter of 52.16 mm. As for the effect of concentration, the 10% concentration was the most inhibitory, with an average diameter of 24.67 mm, while the 0% concentration was the least effective, giving an average diameter of 58.75 mm.

Alcoholic extracts of onion, clove, cinnamon, and rosemary showed varying efficacy in inhibiting the growth of five fungi (*Niger*, *Flavus*, *Tyrus*, *Penicillium*, and *West*), with higher concentrations associated with greater inhibition. Cinnamon and clove showed the highest inhibition levels, especially at 10 mg/ml, with significant sensitivity to yeasts. The statistical differences between concentrations and the diversity of efficacy among plants support the importance of natural active compounds as promising antifungal alternatives to synthetic drugs.

## Discussion

Many toxin-producing fungi have been isolated from maize crops, such as: *Aspergillus flavus*, *Aspergillus tamarii*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Fusarium oxysporum*, *Fusarium proliferatum* and *Penicillium atramentous*. Mycotoxins are natural toxins that are hazardous to health and are produced by some different fungal species and can be found in food. They are carcinogenic, immunosuppressive, and toxic to the liver. The fungus grows on a variety of different crops and foodstuffs including grains, nuts, spices, dried fruits, apples, potatoes and coffee beans, often under poor storage conditions. (Campagnollo et al., 2020) (Zahra et al., 2019)

One type of these toxins is aflatoxins, which are very powerful and broad-acting natural toxins that are produced as secondary metabolites by certain fungi (especially *Aspergillus flavus*, *A. parasiticus*, and *A. niger*). These fungi may grow in dried foods or dried feeds such as grains, seeds, dried fruits, dried meat and fish. There are several types of aflatoxins of varying toxicity: B1, B2, G1, G2, M1 and M2. The most toxic type is aflatoxin B1. Aflatoxins are among the most powerful mutagenic and carcinogenic substances. Some scientists believe that these mycotoxins and some secondary metabolites act as virulence factors for the fungus, meaning that they give it the ability to compete in its surroundings with some microorganisms, or that they increase the resistance of spores to the surrounding environmental conditions (Ismail & Papenbrock, 2015). (Pfliegler et al., 2020)

Clove oil contains eugenol, a compound that disrupts fungal cell membranes and causes oxidative stress within fungal cells, halting vital metabolic processes. It also disrupts fungal cell membranes and inhibits ergosterol synthesis, leading to loss of fungal membrane stability and cell death (ZAHRAOUI,et al. 2025)

Cinnamon, particularly cinnamon and its volatile oils, act as antioxidants and inhibit important enzyme pathways in fungi, contributing to impaired fungal growth and effective suppression. Blank,et al.,2022)(

The varying response of fungi to different plants is attributed to the wide diversity in fungal cell wall composition and metabolic pathways that characterize different fungal species, making some species more susceptible to the effects of natural plant-produced compounds.

The fungal cell wall is primarily composed of basic components such as glucans, chitin, and glycoproteins, and the proportions and compositions of these components vary among different fungal species. These differences directly affect the rigidity of the cell wall and its ability to interact with plant compounds. Cell wall composition plays a key role in determining fungal resistance or sensitivity to natural antifungal compounds.

In addition, the diversity of fungal metabolic pathways contributes to their varying ability to interact and respond to various plant compounds. Processes such as gene duplication and horizontal gene transfer contribute to the production of unique metabolic pathways for each fungal species,

affecting how fungi respond to these natural compounds and their susceptibility to them. (Wisecaver, et al.,2014)(Garcia-Rubio et al .,2019) (Garcia-Rubio et al .,2020)

Thus, these findings confirm the vital role of natural chemical compounds in plant extracts as effective antifungals through multiple mechanisms, such as membrane disruption, enzyme inhibition, and exposure to oxidative stress. This enhances their potential as safe and effective alternatives to chemical drugs in the treatment of fungal infections.

## Conclusion

Several fungal species were isolated from potato and corn chips in, all capable of producing aflatoxins.

Plant extracts (onion, clove, cinnamon, and rosemary) proved effective in inhibiting fungal growth to varying degrees depending on the species and concentration.

The variation in fungal response is due to the diversity of cell wall composition and metabolic pathways among species, which affects their sensitivity to natural compounds.

## Recommendations:

Use plant extracts as safe and effective antifungals as an alternative to preservatives.

Improve storage methods to reduce fungal growth and toxin production in foods.

Conduct further studies to identify effective compounds and develop their practical applications.

Raise awareness about the dangers of mycotoxins and the importance of food safety.

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