Evaluation of Mustard oil and temperature in the toxicity reduction of *Aspergillus flavus* in Walnut fruits

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Abstract

The study aimed to isolate and identify fungi that produce mycotoxins from walnut fruits and the possibility of limiting their presence in the fruits and reducing their toxic effects. The molecular diagnosis in this study of the fungus *Aspergillus flavus* was carried out using the internal transcribed spacer (ITS) genetic marker. As for the results of testing the ability of some *A. flavus* isolates to produce aflatoxins B1 including the ammonia test, the results of this test showed the ability of four out (of six) isolates of *A. flavus* on the production of aflatoxin B1 by changing the color of the base of the coconut medium on which the fungus isolates are grown, with a percentage of 66.66 %. The results of the chemical analysis using TLC technology for *Aspergillus flavus* showed the ability of three isolates to produce aflatoxin B1 out of six isolates of A. *flavus* with a percentage of 50 %. As for the most important results of the effectiveness of mustard oil in inhibiting the growth of the fungus *A. flavus* in walnut fruits, the highest percentage of inhibition was at 15% concentration, which amounted to 57.33%. The most important results of the storage experiment after three months of storage, included the test of the effect of mustard oil and temperature on the growth and density of *A. flavus*. The test was the effect of mustard oil on the reduction ratio of aflatoxin B_1 as it reached the highest percentage of toxin reduction when adding mustard oil only, which amounted to 98.213%, compared to other treatments, the test was the effect of mustard oil and temperature on the amount of aflatoxin B1 toxin, which gave the highest rate when adding *A. flavus* to walnuts, which amounted to 13.425 micrograms/ g,. At a temperature of 5 °C, it gave the lowest rate of aflatoxin B_1 toxin, which was 0.880 $\mu g.gm^{-1}$. The highest rate was 8.620 $\mu g.gm^{-1}$, at 25°C.

Keywords: mycotoxins, walnut fruits, *Aspergillus flavus.*

Introduction

The walnut (*Juglans nigra*) is a northern hemisphere fruit tree with a temperate and subtropical climate (30) and is native to Europe and Asia from the Balkans to southwestern China (15). It is widely cultivated all over the world because of its nutritional value (1). Walnuts are high in mono-saturated fatty acids, vitamins, proteins, and carbohydrates, and as well as it is rich in omega-3. People have been aware of this fruit since the beginning of time. Because its botanical structure is similar to and nutritious to the human brain (29), it is associated with a lower risk of vascular and heart injury (21). During storage with fungal species, including *Asperillus spp*, *Fusarium spp*, and *Pancillium spp*, they produce a wide range of toxic receptors (27). They produce antioxidants against these toxins in addition to their resistance to high temperatures. (34) and many methods have been used to control fungi in the store, including the use of chemicals such as sterilization using carbon dioxide and others(23, 37), The use of chemicals is not without negatives as a result of environmental pollution and the resistance shown by the pest towards these materials Chemicals and damage to human and animal health as a result of the accumulation of these substances in the food chain (33), where plant extracts were used because they have antagonistic activity for many fungi and have preferred characteristics such as their rapid decomposition and lack of impact on human health (17). The use of some essential oils derived from plants such as mustard oil because it has anti-fungal, anti-bacterial, anti-microbial, and food pathogen activity (11 and 8). Therefore, the current study aimed to investigate the fungi contaminating walnut fruits and test the toxins production and study the possible measurements in reducing infection and combined toxicity. It also aimed to confirm the diagnosis of fungi using the Polymerase chain reaction (PCR) technique and testing the extent of contamination of walnut fruits with fungi and testing the extent of susceptibility to fungi.

Materials and Methods

Nut sample collection:

Nut samples were obtained from local markets (American, Iranian and Chinese) for the purpose of obtaining isolates of toxin-producing fungi and then transferred to the Laboratory Plant Diseases at the College of Agriculture - University of Kufa to isolate and identify the fungi accompanying those samples.

Isolation and diagnosis of fungi infecting walnuts

Nus t samples were taken and isolated from them by dilution method and direct cultivation on PDA medium, where the fruits were cut into small pieces (1 cm) and were superficially sterilized with sodium hypochlorite solution 2% concentration for two minutes after which those pieces were washed with sterile distilled water and then placed on filter papers to get rid of free water Then the fruit pieces were planted in plastic dishes (9 cm in diameter) containing the medium of PDA by placing four pieces at a distance of 3 cm from the edge of the dish and a fifth piece in the middle of the dish. (20), and after the end of the incubation period, the growing fungi isolates were purified and then identified based on the taxonomic characteristics mentioned by Pitt and Hocking (24).

The percentages of appearance and frequency of fungi were calculated according the following two equations:

$$\text{Percentage of appearance (\%)} = \frac{\text{The number of samples in which the sex or gender appeared}}{\text{Total number of samples}} \times 100$$

$$\text{Percentage Frequency (\%)} = \frac{\text{The number of isolates of one species}}{\text{The total number of isolates of all fungi}} \times 100$$

Molecular diagnosis of isolates of the fungus *A. flavus*

Molecular diagnosis of the fungus *A. flavus* isolated from walnuts was carried out in the Virology Laboratory/Faculty of Agriculture - University of Karbala. Using the internal transcribed spacer (ITS) genetic marker

Aflatoxin B1 producing by *A. flavus* isolates

Detection of Aflatoxins by ammonia treatment method:

The ability of *A.flavus* isolates isolated from the tested nuts to produce aflatoxin B1 was carried out using (10) method, using coconut medium. In dishes each with a diameter of 9 cm, then 3 dishes were inoculated with tablets of fungi for each isolate, by placing a disk with a diameter of 5 mm from the medium of the PDA grown on The Fungi, at the age of one week, in the center of the dish. A week later, the ability of the mushroom isolates to produce aflatoxins was revealed using a 20% ammonia solution by placing filter papers saturated with ammonia solution in the cover of the dish containing the isolate of the fungi growing in the middle of the coconut. The color of the colony bases from transparent to pink or red indicates that this isolate is capable of producing aflatoxins.

Detection of aflatoxins using Thin Layer Chromatography (TLC):

The isolates of the fungus *A. flavus* on liquid PD medium by placing three tablets of five mm in diameter, one week old, in a flask of 250 mL volume, at a rate of 100 m. L^{-1}. Thereafter the extract was filtered through Whatman No. 4. Then the filtrate was then placed in a separator funnel with a capacity of 250 mL, then 30 mL of chloroform was added to it, then the funnel was shaken gently for 30 seconds while expelling the accumulated gases whenever needed (at least twice) and then left on the holder for one minute in order to The two layers separate, I neglected the upper layer and took the lower layer and repeated the process for three times. Then the filtrate was taken and placed in a clean, sterile flask and placed in the electric oven at a temperature of

40°C until it dried, then dissolved in one ml of chloroform. The presence of aflatoxin, B1 was detected using thin-sheet chromatography (TLC) technology with dimensions of 20-20 cm. The plates were activated in the electric oven was heated at 105 °C for one hour before use (7).

A light straight line was made on the TLC plate at a distance of 1.5 cm from the base of the plate, and 15 µl was taken by capillary tube of the standard toxin AFB1 and placed on the line at a distance of two cm from the left edge of the plate and at a distance of 2 cm from the spot of the standard poison. *A.flavus* at the same distance and in an amount equal to the amount of the standard poison, then the spots were left to dry and then placed in a separation tank containing a separation system consisting of a mixture of chloroform and methanol at a ratio of 2:98 v/v and monitored until the solution reached a distance of approximately two cm from the upper end For the plate, the plates were taken out and dried under laboratory conditions for 5 minutes and then examined under ultraviolet light at a wavelength of 365 nm and the presence of aflatoxin B1 was detected by matching the migration factor Rf and fluorescence color of the standard poison with the color and migration factor of samples of extracts of *A.flavus* isolates (31)

Testing the efficacy of Indian mustard oil extract on the growth of *A.flavus* on PDA medium:

Indian mustard oil was obtained from one of the local markets, so it was added in proportions 5, 10 and 15 mL / 100 mL of PDA medium and poured into the dishes. A fungus from the tested fungi. Three plates of PDA medium (control treatment) were inoculated with PDA discs only on which the fungus *A. flavus* was grown. The dishes were incubated at 25 ± 2 °C for a week. After that, the diameters of *A. flavus* colonies were calculated, and according to the implemented treatments, the inhibition percentage was extracted according to the Abbott equation reported by Shaaban and Al-Mallah (35) where**:**

$$\text{Inhibition}\% = \frac{\text{R1}-\text{R2}}{\text{R1}} \times 100$$

R1 = maximum radial growth of a fungus colony (control treatment).

R2 = maximum radial growth of the studied fungus colony in the treatment dishes.

Evaluation of the efficiency of Indian mustard oil and storage temperature in protecting walnut fruits from infection with the fungus *A. flavus*:

This experiment was carried out to find out the effectiveness of both mustard oil and temperature on protecting walnut fruits from infection with *A. flavus.*

1- Preparation of *A. flavus* inoculum: *A. flavus* inoculum was prepared by growing the fungus on PDA medium for a week under temperature of 30 ± 2°C. Then the fungal spores were harvested by adding 10 mL of sterile distilled water to each of the plates on which the fungus was grown, and then passed A sterile glass rod on the surface of the colonies to facilitate the process of separating spores from the conidia carriers. Then, planktonic spores were collected for each fungus separately and their numbers were calculated using a hemocytometer slide. The fungal spore concentrations were adjusted at (106 spores/mL water).

2- Design of the experiment: The following treatments were applied to walnut fruits, where 10 mL of:

1-Distilled water only 200 gm of nuts
2-10 mL Indian mustard oil 200 gm walnuts
3-The spores of *A. flavus* are stuck in only 200 gm of nuts
4-Suspended *A. flavus* spores + 10 mL of Indian mustard oil 200 gm of nuts

Each treatment was stored in three bags containing each bag (200 gm) for a period of (30, 60, and 90) days at temperatures (5, 15, 25, and 35) °C, after which the contamination percentage was calculated in each of the implemented treatments. Taking 10 g of each of the repeaters of one treatment and placing it in an electric mixer with the addition of 100 mL of sterile distilled water, then it was mixed for 5 minutes, then a series of dilutions was made until 10^{-5}, then one mL of the last dilution was grown on the PDA medium. And three replications for each treatment, and thus all treatments were dealt with, after which the dishes were incubated at a temperature of 25 ± 2 ° C. After that, the number of colonies in each dish was recorded after 3-4 days. Then, the number of active units of the fungus was calculated, according to the coefficients, according to the following equation:

The number of active units = the average number of spores per replicate x the reciprocal of the dilution**.**

The concentrations of aflatoxin B1 were measured by a spectrophotometer, after extracting the mycotoxins from the samples of the implemented treatments, the aflatoxin B1 toxin was extracted by taking 30 gm of walnuts from each treatment and then transferring it to an electric mixer containing 100 mL of chloroform and then mixing the mixture for 10 Minutes after that, the mixture was filtered by filter paper, then the filtrate was taken and placed in a clean and sterile flask and placed in an electric oven at a temperature of 40 ° C until dryness, then it was dissolved in 5 mL of chloroform and then the concentration of the poison was estimated. As for the ochratoxin a poison, it was extracted by taking 30 gm of chloroform. The atmosphere from each treatment and Then to an electric mixer containing 100 mL of the extraction solution consisting of acetonitrile-water (90:10) mL, then mix the mixture for 10 minutes, then filter The extract through what man No. 4. Then the filtrate was placed in a separator funnel with a capacity of 250 mL, then 25 mL of hexane was added to it for the purpose of getting rid of fat (Defatting), Then The funnel was shaken gently for 30 seconds while expelling the accumulated gases whenever needed (at least twice). Then it was left on the holder for one minute in order for the two layers to separate, I neglected the top layer and took the bottom layer, and repeated the process Three times to ensure the removal of fat. Then, 25 mL of distilled water, 8 mL of saturated sodium bicarbonate solution ($NaHCO_3$), and 25 mL of chloroform were added to the lower layer, and after three minutes, the upper and lower layers were separated, keeping the lower layer. The process was repeated twice. Then the extract was transferred to a separating funnel with a capacity of 100 mL, 15 mL of 1-N hydrochloric acid, 20 mL of chloroform, and

20 mL of chloroform were added to it. Shake well and leave the funnel on the holder for one minute. The lower layer was taken and to the upper layer, 20 mL of chloroform was added and extracted again. The two lower layers were collected and passed through filter paper containing a layer of anhydrous sodium sulfate (Na_2SO_4) in order to get rid of the remaining water. Then the filtrate was taken and placed in an electric oven at a temperature of 40 °C until dryness, after which it was dissolved in 5 mL of chloroform (9) and then the poison concentration was estimated by a Spectrophotometer. This method relied on the spectrophotometric estimation, which depends by its nature, on the property of the compound to absorb light in the ultraviolet or infrared wavelengths, where there is a direct proportion between the absorption period and the concentration of the poison. It is possible by drawing a standard curve between the light absorption and the concentration of the poison and extracting the concentration value corresponding to the reading given by the unknown sample. Then the readings were compared with Standard poison concentrations (Supplements 1 and 2), where the wavelengths were 365 and 360 nm for aflatoxin B1 and ochratoxin A, respectively. The reduction ratios were calculated

Using the following equation:

$$\text{Reduction ratio} = \frac{\text{Concentration of control treatment - concentration of the model}}{\text{Control treatment concentration}} \times 100$$

Statistical analysis:

All experiments were carried out in a completely randomized design, C.R.D (Complete Random Design) as one-factor experiments, and the averages were compared according to L.S.D (Less significant differences) method and under a probability level of 0.05 (4).

Results and Discussion

Isolation and identification of fungi accompanying the nut:

The results of isolating fungi from walnuts showed the appearance of five species of fungi. The most common were *A. tubingensis*, *A. flavus*, *Fusarium* sp, *Penicillium* sp, and *Trichoderma* sp. Its frequency rates reached 55.17, 20.68, 13.79, 6.89 and 3.44%, respectively. (Table 1) While the incidence of (100, 66.66, 33.33, 33.33 and 16.66) %, respectively, explains the reason for the dominance of *Aspergillus sp*. The ability to withstand drought and high humidity (2), and withstand high temperatures up to 50 °C (12).

Table 1. Percentages of the frequency and appearance of fungi isolated from walnut

fungus type	(%) frequency	(%) apperance
A. tubingensis	55.17	100
A. flavus	20.68	66.66
Fusarium	13.79	33.33
Penicillium	6.89	33.33
Trichoderma	3.44	16.66

Molecular diagnosis of *A. flavus* isolates

The results of the molecular diagnosis of the pathogenic fungus *Aspergillus flavus* indicated that this isolate was established in the Neighbor-joining tree and in the National Center for Biotechnology Information (NCBI) under the entry (Accession number ON394601.1.) Based on the sequences of its nitrogenous bases for the ITS-rDNA region as well as the sequences of global strains of the same pathogenic fungus were obtained from Gen Bank Data Repository. The genetic distances were calculated using the neighbor-joining method.

Testing the ability of some isolates for *A. flavus* to produce aflatoxins B1:

Ammonia test:

The results of this test showed the ability of 4 out of 6 isolates of *A. flavus* to produce aflatoxin B1 by changing the color of the base of the coconut medium on which the fungus isolates were grown, with a percentage of 66.66 %. The isolates that produced the most aflatoxins were Af4, while isolates Af2 and Af5 showed a medium ability to produce aflatoxins, and the rest of the isolates were weak in their production of aflatoxins (Table 2).

The fungal strains vary in their ability to secrete aflatoxin, some of them may excrete aflatoxin toxins, while there are other strains that secrete more than one type of mycotoxin depending on the type of fungal strain (16). The biological basis of the ammonia test depends on the production of a group of yellow dyes with different chemical structures that are intermediate compounds in the aflatoxin synthesis pathway, and that these dyes turn red or purple when combined /*with a basic solution - such as ammonium hydroxide, potassium or sodium bicarbonate. This result is also in line with what was found by (5) that the percentage of aflatoxin-producing *A. flavus* isolates isolated from field pistachio seeds was 30.8%, while reported that 86% of the aflatoxin-producing *A. flavus* Isolates of *A. flavus* are able to infect field pistachios and contaminate them with aflatoxins. (3) Indicated that 59% of *A. flavus* isolates isolated from some foods in Baghdad city are capable of producing aflatoxins. The difference in the ability of isolates to produce aflatoxin quantitatively and qualitatively may be due to the different genetic content of the strains, and this explains the gradient in the red color. (26).

Table 2. Testing the ability of some isolates of *A. flavus* to produce aflatoxin B1 in medium coconut (CEA)

fungul isolation	The ability to produce aflatoxin B1
Af1	+
Af2	++
Af3	-
Af4	+++
Af5	++
Af6	-

(+): the color of the middle base changed to pink, (-): the color of the middle base did not change.

Detection using thin sheet chromatography (TLC) method:

The results of this test showed the ability of some isolates of *A. flavus* to produce aflatoxin B1, whereas the test showed the ability of 3 isolates to produce aflatoxin B1 out of 6 isolates of *A. flavus* at a percentage of 50%, as in (Table 3). The fungus isolates varied in their toxin production, and isolate AF4 was the most toxin-producing isolate based on the intensity of its fluorescence. And Af5 and Af2 are the least productive of aflatoxin production. These results initially agree with what one study indicated about the ability of 75% of *A. flavus* isolates to produce aflatoxin B1. And an approach to what was mentioned (14). This indicated that 38.88% of *A. flavus* isolates isolated from walnuts are able to produce aflatoxin B1. The difference in the ability of isolates to produce aflatoxins B1 may be attributed to the presence of genetic differences between the fungal isolates (18). It is noted that the percentage of isolates producing aflatoxin B1, which were detected by this technique, is less than the number of isolates producing aflatoxin B1 using the ammonia reaction technique. Accordingly, the thin plate chromatography method is the most accurate method for determining the isolates producing mycotoxins in general, including aflatoxin B1.

Table 3. Testing the susceptibility of a number of *A. flavus* isolates to produce aflatoxin B1 isolated from walnuts by thin plate chromatography (TLC) method

fungul isolation	The ability to produce aflatoxin B1
Af1	-
Af2	+
Af3	-
Af4	+++
Af5	++
Af6	-

(+) aflatoxin B1-producing isolate (-) non-aflatoxin B1-producing isolate

Evaluation of the effectiveness of different concentrations of mustard oil in inhibiting the Qatari growth of the fungus *A. flavus*:

The results of the experiment showed the treatment of nuts contaminated with the fungus *A. flavus* with mustard oil helped it to inhibit the growth of the fungus *A. flavus*, as the percentage of inhibition increased by increasing the concentration and the highest percentage of inhibition was at the concentration 15%, which amounted to 57.33%, and the inhibition percentage in concentrations 5, 10 and 15 (48.00, 52.00 and 57.33%), respectively, as in the (table 4) This is similar to what previous studies indicated to the effectiveness of many medicinal plants and their volatile oils in inhibiting toxin-producing fungi (28).

Table 4. Effect of different concentrations of mustard oil on the growth of *A. Flavus* grown on PDA

Transactions	retarding percentage%	Colonies diameter average (cm)
PDA. Comparison	0.00	7.5
5	48.00	3.9
10	52.00	3.6

15	57.33	3.2
L.S.D.(0.05)	1.631	0.1883

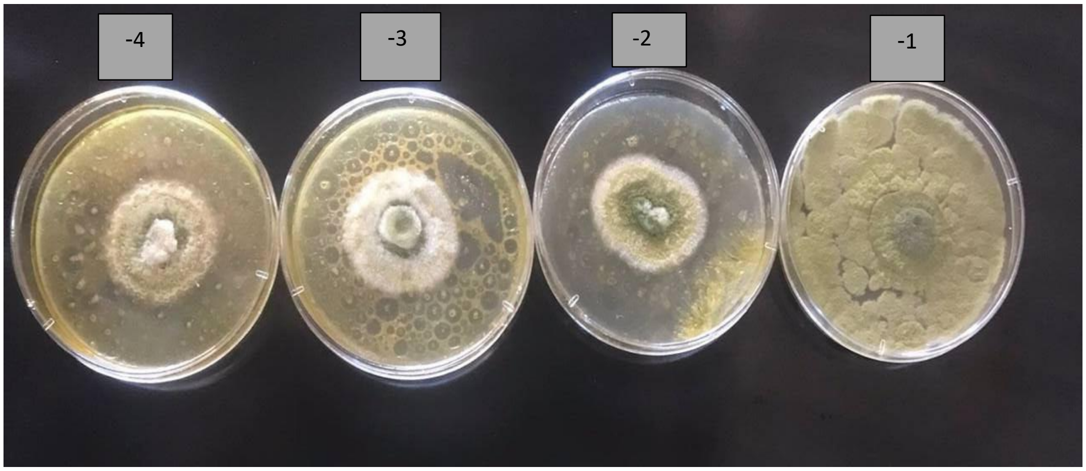

Figure 1. shows the evaluation of the effectiveness of different concentrations of mustard oil in inhibiting the growth of the fungus *A. flavus*, which are three different concentrations (5%, 10% and 15%). No. (1) represents the comparison, which is *A. tubingensis* only, and No. (2) represents A concentration of 5% and number (3) represents a concentration of 10% and number (4) represents a concentration of 15%.

Effect of mustard oil and temperature on the growth and density of *A. flavus* after three months of storage.

The results of this test showed significant differences in the growth rate of the number of reproductive units of *A. flavus*_spores, as it gave the highest growth rate when *A. flavus* was added to the nut, which amounted to 3.200 x 10^{-5} spores/gm compared to the control treatment (0.25 x 10^{-5} spores. gm^{-1}). In Table (5), while the mustard oil reduced the numbers of microbial units of the fungus, where the treatment of *A. flavus*_+ mustard oil gave 0.575 × 10^{-5} spores / gm^{-1}, compared with the treatment of adding fungus only, which amounted to 3.200 × 10^{-5} spores. Gm-1, while the oil treatment was given Mustard only had the lowest growth rate in the number of reproductive units, which amounted to (0.000 x 10^{-5} spores. gm^{-1}) compared to the control treatment (0,255 x 10^{-5} spores. gm^{-1}). Whereas, the addition of distilled water gave only 1.425 x10^{-5} spores / g, and the reason is the presence of erucic acid in mustard seed oil, which plays a role in phytoremediation and antioxidants (36). The table also indicates that there are significant differences in the effect of temperature on the number of reproductive units of *A. flavus*, so the storage treatment at a temperature of 35 °C gave the lowest rate in the number of reproductive units of the fungus, which amounted to 0.575 × 10^{-5} spores. gm^{-1} compared to the other treatments (5, 15, 25). mL, which gave (1.080, 1.140, 1.560 × 10^{-5} spores. g), respectively. The highest rate in the number of reproductive units of the fungus was (1,560 × 10^{-5} spores. gm^{-1}) at 25 °C compared with other treatments, so crop losses were recorded from 20% to 50% in developing countries due to inappropriate storage practices (22).

Table 5. Effect of mustard oil and temperature on the growth and density of *A. flavus* after three months

Transactions	Number of reproductive units of spores (spore/gm) x 10^5				The average
	Temperatures				
	°م5	°م15	°م25	°م35	
Comparison	0.0	0.4	0.2	0.3	0.225
distilled water	0.4	1.6	2.6	1.1	1.425
Mustard oil	0.0	0.0	0.0	0.0	0.000
A. flavus	4.2	2.9	4.6	1.1	3.200
A. flavus +Mustard oil	0.8	0.8	0.4	0.3	0.575
The average	1.080	1.140	1.560	0.575	1.080

L.S.D. 0.05 coefficients = 0.0715, temperatures = 0.0639, coefficients + temperatures = 0.1429

Effect of mustard oil and temperature in reducing the toxicity of aflatoxin B1 produced by *A. flavus* after three months.

This test showed the effect of mustard oil on the reduction ratio of aflatoxin B1 when it gave (mustard oil, *A. flavus*, mustard oil + *A. flavus*) (98.213, 0.000, 71.188) % respectively, as indicated in Table (6), where the highest percentage of toxin reduction when adding mustard oil only, which amounted to 98.213% compared to other treatments, and the lowest percentage of toxin reduction in the treatment of adding *A. flavus* only, which amounted to (0.0000%) compared to other treatments because it has many The characteristics that make mustard a valuable botanical medicine (19).The table also indicates that there are significant differences in the effect of temperature on the percentage of toxin reduction produced by the fungus. % compared to the other treatments (5, 15, 25 °C) which gave (42.855, 41.927, 42.953) %, respectively, and the highest rate was 42.953% at a temperature of 25 °C compared to other treatments, due to the conditions that promote the growth of fungi always may not lead to the production of mycotoxins. In general, it water activity above 0.78, relative humidity between 88% and 95%, and temperature between 25 and 30 °C are favorable conditions for the growth of fungi and production of mycotoxins (32).

Table 6. Effect of mustard oil and temperature in reducing the toxicity of aflatoxin B1 produced by *A. flavus* after three months

Transactions	% reduction ratio				The average
	Temperatures				
	5م°	15م°	25م°	35م°	
Comparison	0.0	0.0	0.0	0.0	0.00
Mustard oil	100.0	92.85	100.0	100.0	98.213
A. flavus	0.0	0.0	0.0	0.0	0.0000
A. flavus + Mustard oil	71.42	74.86	71.81	66.66	71.188
The average	42.855	41.927	42.953	41.665	42.350

L.S.D. 0.05 Transactions = 0.5109, temperature = 0.5109, Transactions + temperature = 1.0219

Effect of mustard oil and temperature on the amount of aflatoxin B1 microgram/gm produced by *A. flavus* after three months of storage:

The results of this test showed significant differences in the amount of aflatoxin B1 toxin produced by *A. flavus* fungus, as it gave the highest rate when adding *A._flavus* to walnuts, which amounted to(13.425 µg.g^{-1}) compared to the control treatment, which amounted to (2.708 µg.g^{-1}) As indicated in (table 7) While mustard oil reduced the amount of aflatoxin B1, the treatment of *A. flavus* + mustard oil gave 3.800 µg.g^{-1} compared with the treatment of adding *A. flavus* only, which amounted to (13.425 µg.g^{-1}), while the treatment of adding *A. flavus* only gave (13.425 µg.g^{-1}). The addition of mustard oil only had the lowest rate, which amounted to (0.075 µg/gm) compared to the control treatment (2.708 µg. g^{-1}). While the treatment of adding distilled water gave only 8.708 µg/gm. This is similar to what (25) said that mustard oil is used as a treatment and antimicrobial and is of plant origin to inhibit fungal and bacterial microbes. The table also indicates that there were significant differences in the effect of temperature on the amount of aflatoxin B1 toxin produced by A. flavus, so the storage treatment at a temperature of 5 °C gave the lowest average in the amount of aflatoxin B1 toxin, which was 0.880 µg.g^{-1} compared to the other treatments (15, 25, 35) m ° which gave (8.307, 8.620, 4.760) µg.g^{-1} respectively. And the highest rate was (8.620 µg. g^{-1}), at a temperature of 25°C compared with the other treatments. Because the production of mycotoxins from *Aspergillus*, *Pencillium* and *Fusarium* depends on the biochemical, genetic and physiological characteristics of the fungal strains and also on external factors such as temperature, humidity and specific environments (12).

Table 7. Effect of mustard oil and temperature on the amount of aflatoxin B1 microgram/gm produced by *A. flavus* after three months of storage

Transactions	The amount of aflatoxin B1 µg/gm				The average
	Temperatures				
	°م5	°م15	°م25	°م35	
Comparison	0.0	4.2	5.4	1.2	2.708
distilled water	2.6	14.1	9.0	6.6	8.075
Mustard oil	0.0	0.3	0.0	0.0	0.075
A. flavus	1.4	18.3	22.0	12.0	13.425
A. flavus + Mustard oil	0.4	4.6	6.2	4.0	3.800
The average	0.880	8.307	8.620	4.760	5.617

L.S.D. 0.05 coefficients = 0.3742, temperatures = 0.3347, coefficients + temperatures = 0.7484

Conflict of interest

The authors have no conflict of interest.

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