STUDY OF *Bacillus licheniformis* BACTERIA AS A BIO-CONTROL AGENT IN REDUCING AFLATOXIN B1 TOXICITY IN DRIED GRAPE SAMPLES

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ABSTRACT

This study was aimed to isolate and identify fungi contaminating raisin samples and evaluate the effectiveness of bacteria in inhibiting fungal growth and reducing toxin levels. The isolation results the fungus *A. flavus* was the most frequent and appeared in the samples, reaching 54.5 and 44.4%, respectively. The results showed that all isolates of *A. flavus* had the ability *to* produce aflatoxin B1, as confirmed by the ammonia vapor test. The isolate with the highest toxin production was identified through nucleotide sequences deposited in the World Genomic Organization's Gene Bank under accession number OR192858. The results demonstrated the ability of the live bacteria *Bacillus licheniformis* to inhibit fungal growth, reaching 94.4%, compared to 72.56% for the killed bacterial filtrate. The toxin concentration was 62.11 ppb in the toxin-producing isolate treated with bacteria, while it was 285.866 ppb in the toxin-producing isolate without bacteria. An 85.65% reduction in toxin levels was observed compared to the control, which registered 0%, with a toxin concentration of 41.08 ppb in the bacteria-treated toxin-producing isolate, while it was 285.866 ppb in the untreated toxin-producing isolate.

Keywords: fungal toxins, *Aspergillus flavus*, ammonia, secondary metabolites, hplc

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دراسة بكتيريا *Bacillus licheniformis* كعامل مقاومة حيوية في اختزال سم الافلاتوكسين B1 في عينات العنب المجففة

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المستخلص

هدفت الدراسة الى عزل وتشخيص الفطريات الملوثة لعينات الزبيب وتقييم فعالية البكتيريا في تثبيط الفطر واختزال السم. أظهرت نتائج العزل ان الفطر *A.flavus* كان الاكثر تردد وظهورا في العينات اذ بلغت 54.5 و44.4 % على التتابع . بينت النتائج قدرة جميع عزلات الفطر *Aspergillus flavus* على افراز سم الافلاB1 باختبار بخار الامونيا وتم تشخيص العزلة الاكثر انتاجا للسم وودعت التتابعات النيوكليوتيدية في المنظمة العالمية لبنك الجينات تحت رقم الانضمام OR192858 .اوضحت النتائج قدرة البكتيريا الحية *Bacillus licheniformis* في تثبيط نمو الفطر اذ بلغت 94.4 % في حين بلغت 72.56 لراشح البكتيريا المقتولة . ان تركيز السم بلغ 62.11 ppb في معاملة العزلة المنتجة للسم مع البكتيريا بينما بلغ 285.866 ppb في العزلة المنتجة للسم ،في حين سجلت نسبة اختزال 85.65% مقارنة بمعاملة السيطرة التي سجلت 0% وبلغ تركيز السم 41.08 ppb في العزلة المنتجة للسم المعاملة_مع البكتيريا بينما بلغت 285.866ppb في معاملة العزلة المنتجة للسم .

الكلمات المفتاحية: السموم الفطرية، *Aspergillus flavus* ، الامونيا، مركبات الايض الثانوية،hplc

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INTRODUCTION

Mycotoxins are secondary metabolites produced by various fungi, most of which belong to the genera *Aspergillus spp*, *Penicillium spp*, and *Fusarium sp*p (28). They are characterized by their low molecular weight and are known for their harmful effects even at low concentrations (29). *Aspergillus flavus* is a widely distributed filamentous fungus in nature with the ability to cause diseases in both humans and animals. It is known for its diversity of species, production of various substances and toxins(27) .The fungus is characterized by its ability to produce toxins and is among the most significant producers of mycotoxins worldwide. It produces AFB1 and AFB2, which impact many food items. Storage conditions at high temperatures with humidity contribute to the proliferation of fungi and their release of mycotoxins, affecting various agricultural products, including dried fruits such as raisins, peaches, figs, and apricots. These dried fruits represent a rich source of minerals, vitamins, and fibers. Raisins, being a dried fruit, are susceptible to contamination by various fungi, including *Aspergillus* species, and are affected by mycotoxins. Among the mycotoxins associated with dried fruits, aflatoxins are prevalent, along with ochratoxin and patulin. Due to the significant risks associated with aflatoxin mycotoxins and their acute and chronic toxic effects, they have garnered considerable attention from organizations such as the World Health Organization (WHO), the European Food Safety Authority (EFSA), and the Food and Agriculture Organization (FAO). Diagnosed aflatoxins B1, B2, G1, and G2, which are considered among the most dangerous types of toxins for humans and animals and are present in most major food crops (22,33). Aflatoxins are classified as carcinogenic substances and are implicated in causing genetic mutations, immunosuppression, anemia, and an increased likelihood of contracting viral hepatitis (9,34). Due to the danger resulting from the contamination of food substances with these toxins, researchers have turned to using highly efficient methods and environmentally friendly substances to prevent the growth of toxin-producing fungi or reduce and eliminate them from food. This includes the use of plant extracts, chitosan, and nanomaterial as they are environmentally friendly, cost-effective, and highly efficient. Examples include nanoscale algae and the biosynthesis of silver nanoparticles by the fungus *A. niger* and the bacterium *Bacillus licheniformis*, known for their ability to inhibit fungal growth. These microorganisms also have the capability to produce various enzymes such as chitinases, glucanases, proteases, and lipases, which work to break down the cell walls of pathogenic fungi (4,6,10,11 ,20,35). The mechanism of bacteria in inhibiting pathogenic microorganisms lies in their ability to deplete iron from fungi, reducing their pathogenicity. Additionally, bacteria have the capacity to produce various compounds and induce systemic resistance in plants (24). Therefore, the study aimed to evaluate the effectiveness of the bacteria in preventing the fungus *A. flavus* from producing and reducing aflatoxin contamination in dried grape fruit.

MATERIALS AND METHODS

Sample collection: Samples of raisins were collected during the year 2022 from local markets in the city of Baghdad. Each sample consisted of 300 grams, with three replicates for each sample at an average of 100 grams per replicate. The samples were sourced from various locations in Iraq, Syria, Iran, Turkey, and the Czech Republic. They were placed in polyethylene bags, labeled with the sampling location and collection date, and brought to the laboratory for laboratory experiments.

Isolation of accompanying fungi in raisins and their identification: The fungi were isolated from raisin fruit by cutting it into small pieces measuring 0.5 cm. The pieces were then surface-sterilized with a 1% sodium hypochlorite solution for 2 minutes, followed by three rinses with sterile distilled water. The pieces were dried using sterile filter paper (26). Subsequently, the sections were transferred to Petri dishes containing prepared and sterilized Potato Dextrose Agar (PDA) medium. The dishes were autoclaved at a temperature of 121°C and a pressure of 1.5 kg/cm^2 for 15 minutes and then allowed to cool slightly in the isolation room (hood). Antibiotic streptomycin sulfate was added to the medium at a rate of 50 mg L^{-1} and

thoroughly mixed to ensure homogeneity before pouring into the plates (3, 5). Then, they were poured into 9 cm diameter Petri dishes, with 4 pieces per dish and 30 dishes per sample, at a rate of 3 replicates with 10 dishes for each replicate. The plates were incubated for 3 days at a temperature of 25 ± 2°C. Isolated fungi were purified and identified based on their visual characteristics, including colony appearance, morphology of spores, and conidiophores, using taxonomic keys (27 , 38). Isolates belonging to the *A flavus* fungus were stored at a temperature of 4°C. The percentage of occurrence and frequency were recorded to determine the most frequently appearing fungus according to the following equations:

$$\textbf{Occurrence } \% = \frac{\textbf{Number of times the species or genus appeared}}{\textbf{Total number of samples}} \textbf{ x 100}$$

$$\textbf{Frequency } \% = \frac{\textbf{Number of colonies of the species or genus}}{\textbf{Total number of colonie}} \textbf{ x100}$$

Detection of fungal isolates producing aflatoxin B1: Two methods were used to detect the isolates' ability to produce aflatoxin B1 . The first method involved the use of ammonia vapor (specific detection). The isolates were activated on a solid coconut agar (COA) medium, prepared by adding 100 grams of shredded commercial coconut to 300 ml of distilled water and leaving it on the heat for 15 minutes. After that, the solution was filtered through a cloth (gauze), and completed to a volume of 600 ml by adding deionized water. Agar was added at a concentration of 1.5%, and the medium was sterilized using an autoclave at a temperature of 121°C and a pressure of 1.5 kg/cm² for 20 minutes. After sterilization, the medium was cooled, and the antibiotic streptomycin sulfate was added at a concentration of 50 mg L^{-} to prevent bacterial growth . The medium was poured into plates, inoculated with the fungus, and incubated for five days. A filter paper saturated with a 20% ammonia solution was placed on the lid of the petri dish. The plates were then returned to the incubator at a temperature of 25°C ± 2, inverted, and examined after one hour and 24 hours of incubation. The observation focused on the color change of the agar medium from white to pink, indicating the isolates' ability to produce AFB1 .The second method involved using Yeast Extract and Sucrose broth medium (YES). The YES medium was prepared by dissolving 14.6 grams of yeast extract, 133 grams of sucrose, 1.33 grams of K_2HPO_4, 0.33 grams of $MgSO_4$, 3.33 grams of peptone, and 3.33 grams of sodium chloride in one liter of deionized water. Each 100 ml of the solution was placed in a 250 ml glass flask, sterilized with a autoclave at a temperature of 121°C and a pressure of 1.5 kg/cm² for 20 minutes. After sterilization, the medium was cooled, and the antibiotic tetracycline was added at a concentration of 250 grams per liter. Then, the flasks were inoculated by placing three discs measuring 0.5 cm in each flask. These discs were taken from the *A. flavus* isolate that appeared dark in the ammonia vapor test. The flasks were incubated for 14 days at a temperature of 25°C ± 2. Afterward, aflatoxin B1 was extracted, and its concentration was determined using High-Performance Liquid Chromatography (HPLC) technique (9),using High-Performance Liquid Chromatography (HPLC) in the laboratories of the Ministry of Science and Technology – Department of Environment and Water. The analysis was conducted using a German-made SYKAMN High-Performance Liquid Chromatography system. The mobile phase consisted of acetonitrile: distilled water (70:30), and the separation column used was C18 – ODS (25 cm * 4.6 mm). Fluorescence detection (ex=365nm, em=445nm) was employed to detect fungal toxins. The flow rate of the mobile phase was 0.7 ml/min.

The molecular diagnosis of the most toxin-producing *A. flavus* isolate: The DNA was extracted from the pure fungal isolate of *A. flavus* that exhibited the highest production of AFB1 toxin. The fungus was cultured on Potato Dextrose Agar (PDA), and after 4 days, the fungal mycelium was collected in sterile plastic tubes. These tubes were stored in the freezer until DNA extraction was performed using specialized kits from the American company ZYMO, following the company's recommendations and standard procedures.

Amplification of the nucleic acid for the pathogenic agent: Polymerase Chain Reaction (PCR) technology was employed to amplify the DNA of the isolated pathogenic agent from raisin fruit using the primers ITS1 and ITS4 (43), manufactured by Integrated DNA Technology (Table 1). The PCR amplification results were sent to Macrogen Company for the purpose of determining the nucleotide sequence of the amplified DNA of the pathogenic fungal isolate, and it was subsequently deposited in the gene bank.

Preparation of bacterial nanofilter

The bacterial inoculum was prepared by adding 4 grams of Nutrient Broth liquid medium to 250 ml of distilled water in a 500 ml glass flask. It was then sterilized using an autoclave at a temperature of 121ºC and a pressure of 1.5 kg/cm². The characterized bacterial isolate (*Bacillus licheniformis*) was inoculated by taking a sample from the growing isolate on nutrient agar medium aged 24 hours. The nutrient broth medium was inoculated, and it was incubated for two days. Subsequently, the bacterial nanofilter was exposed to an ultrasonication device , and the volume of the nanoscale particles produced was measured using an Atomic Force Microscope (AFM).

Table 1. The nucleotide sequence in the primers used for amplifying fragments of the DNA of *A. flavus*

Primer	Sequence	Tm (ºC)	GC (%)	Product size
Forward	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	650 base pair
Reverse	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41 %	

Testing the antagonistic ability of bacteria against *A. flavus*: The identified and registered bacteria were obtained from the gene bank. OL442684.1 (7) (Study accepted for publication) were obtained for this research. The bacterial inoculum was prepared by adding 4 grams of nutrient broth liquid medium to 250 ml of distilled water in a 500 ml glass flask. It was then sterilized using an autoclave . The characterized bacterial isolate was inoculated by taking a inoculium from the growing isolate on nutrient agar medium aged 24 hours. The nutrient broth medium was inoculated, and it was incubated for two days. Different bacterial treatments were used, including live bacteria, heat-treated bacteria (using autoclaving), bacteria filtrate (treated with a centrifuge), live bacteria filtrate, heat-treated bacteria filtrate, and nano-bacterial filtrate. Each treatment involved adding 1 ml of the bacterial inoculum, grown in Nutrient Broth medium for 48 hours, to plates containing PDA medium (15ml) before solidification. The plates were then gently shaken to evenly distribute the bacterial inoculum with the medium. The plates were inoculated with a 0.5 cm disc from the edge of a 3-day-old *A. flavus* fungal colony, and the same method was applied for control plates without the addition of bacterial inoculum. The plates were incubated at 25±2°C for 6 days, and the inhibition percentage was calculated according to the following equation: (14)

$$\%I = \frac{C - T}{C} X100$$

I: Inhibition , **C:**Diameter of growth in the control. , **T:** Diameter of growth in the treatment

Studying the secondary metabolites produced by bacteria: The live bacteria (which demonstrated the highest effectiveness in inhibiting the fungus compared to other bacterial treatments) were analyzed for secondary metabolites using the GG Mass detector at the Ministry of Science and Technology (16,19).

Test of the bacteria's effectiveness in preventing the fungus from producing aflatoxin: The experiment was conducted by surface sterilizing 600 grams of raisins with a 1% sodium hypochlorite solution for 2 minutes, followed by rinsing with sterile distilled water three times. The dried pieces were then distributed in 250 ml glass flasks, sterilized with autoclave at 121°C and a pressure of 1.5 kg/cm² for 20 minutes. After

the sterilization period, the following treatments were applied:

1-The first treatment involved adding 3 discs measuring 0.5 cm from the edge of a 5-day-old *A. flavus* fungus colony cultured on PDA medium.

2-The second treatment involved treating the raisins with live bacteria. The bacterial inoculum was prepared by adding 4 grams of nutrient broth liquid medium to 250 ml of distilled water in a 500 ml glass flask. It was sterilized using an autoclave at 121°C and a pressure of 1.5 kg/cm². The identified bacterial isolate was inoculated by taking a swab from a 24-hour-old non-viable isolate on nutrient agar medium. After that, the nutrient broth medium was inoculated and incubated for two days. The medium was then inoculated with the isolate producing the toxin and left for 21 day.

Determining the efficiency of bacteria in aflatoxin reduction: The medium was prepared following the same steps as in the previous paragraph, and the following treatments were applied:

1.The first treatment involved adding 3 discs measuring 0.5 cm from the edge of a 5-day-old *A. flavus* fungus colony cultured on PDA medium.

2.The second treatment involved treating the fruit with the isolate producing the toxin and leaving it for 21 days. Afterward, the fruit was autoclaved and, upon cooling, treated with live bacteria for 21 days. After the designated period, AFB1 toxin was extracted, and the reduction percentage was calculated.

Extraction of aflatoxin B1 (AFB1)

After completing the incubation period , 50 grams of raisins were taken and blended with 150 ml of chloroform: water solvent (90:10, v/v) for 30 minutes. The mixture was then filtered using Whatman No.1 filter paper, and the extraction was repeated with 75 ml of the same solvent. The filtrate was collected in glass bottles and evaporated in a water bath at 55ºC until dryness. The sample was stored in the freezer until further experiments. Thedetection and estimation of the AFB1 toxin concentration were performed using High Performance Liquid Chromatography (HPLC) in the laboratories of the Ministry of Science and Technology – Department of Environment and Water . The reduction percentage of AFB1 was calculated using the following equation:

$$\%\textbf{AFB1 R} = \frac{\boldsymbol{C - T}}{\boldsymbol{C}} \textbf{ X 100}$$

R:Reduction . , **C:** Concentration of AFB1 in control. , **T:** Concentration of AFB1 in treatment.

RESULTS AND DISCUSSION

The results of the isolation and identification of fungi accompanying the raisin samples revealed the presence of a group of genera, including *Alternaria sp*., *A. flavus*, *A. nig*er, *Penicillium sp*., and *Rhizopus sp.* The results in Table (2) showed that the fungus *A. flavus* achieved the highest percentage of frequency, recorded at 54.5%, while it reached 45.4% for both *Alternaria sp*. and *A. niger*. *Penicillium s*p. followed with a percentage of 36.3%, and *Rhizopus sp* with 18.1 %.

Table 2. Percentage of Frequency for Isolated Fungi

	Fungi	Frequency %
1	*Alternaria sp*	45.4
2	*A. niger*	45.4
3	*A. flavus*	54.5
4	*Penicillium sp*	36.3
5	*Rhizopus sp*	18.1
	L.S.D	0.819

The results illustrates the percentage of occurrence for the isolated fungi, ranging from 4.4% to 44.4%, with significant differences between them. The percentage of appearance for the fungus *A. flavus* was the highest at 44.4%, followed by 24.4% for *A. niger*, 15.5% for *Alternaria sp*., 11.1% for *Penicillium sp*., and the fungus *Rhizopus* recorded the lowest percentage of appearance at 4.4%. The fungi that appeared during this study are consistent with numerous studies confirming the presence of these fungi in dried fruit samples as a nutritional and carbon source for many fungi (32). Additionally, the high occurrence of *A. flavus* fungi in grains was noted by (15), recording a frequency rate of 36% in their study. (18) reported a frequency rate of 44.78% for *A. flavus* in wheat samples. (13) mentioned the contamination of rice grains with *A. flavus*. Furthermore, (12) confirmed the isolation of *A. flavus* from wheat grains

Detection of aflatoxin-producing fungal isolates: The results of the detection of fungal isolates for aflatoxin production revealed the

ability of these isolates to produce toxins using the ammonia vapor method, with variations in time and intensity of color on the inverted plate, as shown in Figure(1). All isolates demonstrated their ability to produce toxins compared to the control treatment representing no treatment with ammonia. The time of color appearance varied between one hour to 24 hours for the tested isolates. (41) explained that the color gradient depends on the aflatoxin concentration, defining dark red as highly toxic with a toxin concentration of greater or around 2000 ppb. While, light red indicates toxicity with a toxin concentration ranging from 501 to 2000 ppb, and low-toxic or non-toxic isolates are characterized by a creamy color (≤20 ppb). The rapid response may be attributed to genetic variations among *A. flavus* isolates producing AFB1. (13) identified nine out of eleven *A. flavus* isolates capable of aflatoxin production The results showed that the quantitative estimation of aflatoxin B1 by HPLC technique for the most effeciact fungal isolate that appeared the fastest was 268.4 ppb when grown on liquid yeast medium. Research confirms that yeast liquid medium is suitablefor aflatoxin B1 production. The high production in this medium is attributed to the presence of sugars, especially sucrose, which stimulates *A. flavus* to increase toxin production, along with the optimal incubation temperature for toxin production (30). (44)found that sucrose, maltose, and glucose enhance the biosynthesis of aflatoxins in food environments, especially in liquid medium. The temperature range of 28-30 °C is optimal for aflatoxin production, and the fungi's toxin-producing ability decreases at higher temperatures (31 ,46).

Molecular diagnosis of the fungal isolate with the highest AFB1 toxin production

The results of agarose gel electrophoresis of the extracted DNA from the fungal isolate with the highest AFB1 toxin production, as shown in Figure 1, revealed the presence of a single band with a molecular weight of 650 bp (Figure 2).

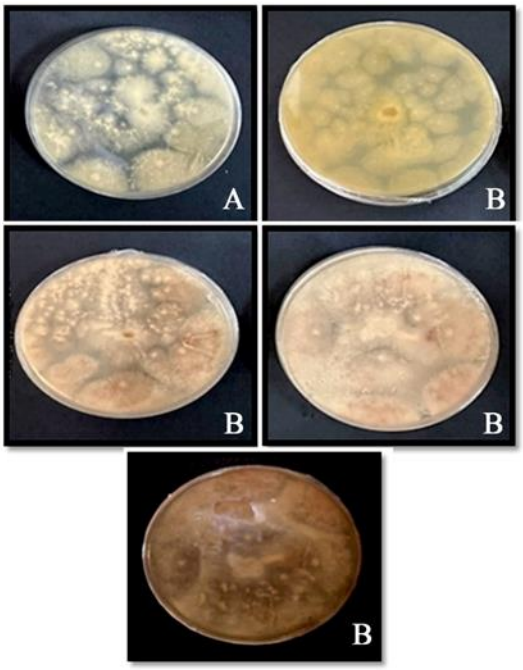

Figure 1. Isolates of *A. flavus* Producing Aflatoxin B1 on Coconut Medium Using Ammonia Vapor

A: Control , B: Fungal Isolates Producing Aflatoxin B1 Molecular diagnosis of the fungal isolate with the highest AFB1 toxin production

The results of agarose gel electrophoresis of the extracted DNA from the fungal isolate with the highest AFB1 toxin production, as shown in Figure(1), revealed the presence of a single band with a molecular weight of 650 bp (Figure 3). This result was obtained using the internal transcribed spacer (ITS) cloning primers ITS1 and ITS4. These findings confirm the efficacy of these primers in amplifying the ribosomal DNA (rDNA) of the fungus *A. flavus.*

Amplification of the nucleic acid for the pathogenic agent: The nucleotide sequencing results in Table (3) shows that the *A. flavus* isolate showed a 99% similarity with international isolates available in the NCBI global gene bank. One site of transition-type variation and one site of transversion-type variation were found. Nucleotide sequences for the fungus were submitted to the International Gene Bank, and accession number OR192858 was obtained, establishing it as a reference for Iraq, the Middle East, and globally

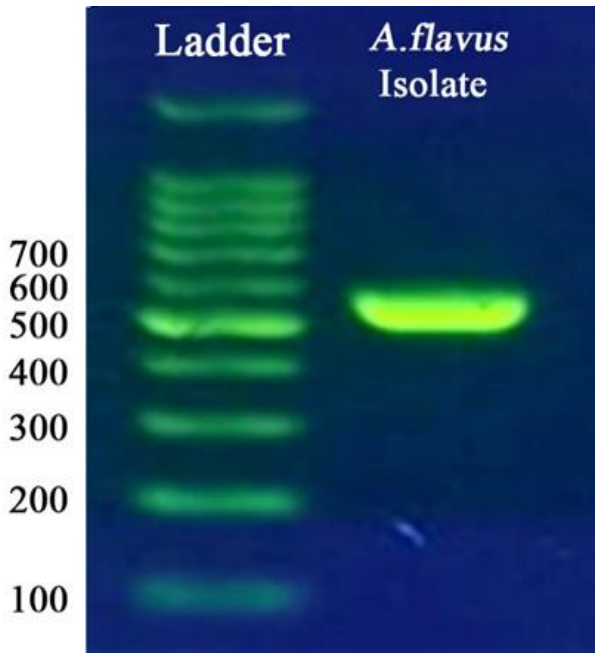

Figure 2. Electrophoresis Image for the Fungal Isolate of *A. flavus*

Preparation of Nanobacterial Filtrate

The results of the bacterial filterate examination revealed that the particle size of the filter reached 31.84 nanometers when examined with an Atomic Force Microscope (AFM).

Antagonistic Test of Bacteria against *A. flavus:* The results in Table (4) demonstrate the effectiveness of all treatments in inhibiting the growth of *A. flavus*, with significant differences among them. The live bacteria treatment achieved the highest inhibition rate at 94.4%, while the dead bacteria treatment reached 92.36%, and the live bacterial filterate recorded 88.7%. On the other hand, the dead bacterial filterate and the nanobacterial filterate showed inhibition rates of 72.56% and 89.4%, respectively. (37) mentioned that bacteria have the ability to produce secondary metabolites that inhibit fungal growth, reducing toxins by up to 94.7%, such as aflatoxin B1 (46). (17) highlighted the bacteria's capability to produce volatile compounds effective against aflatoxin and ochratoxin toxins produced by fungi, along with their potential to produce enzymes and antimicrobial compounds (42). (23) found that *Bacillus licheniformis* bacteria exhibited inhibition rates of 62%, 60%, 53%, 50%, 49%, 47%, and 44**%** respectively against fungi *A. carbonarius*, *Penicillium verrucosum*, *A. niger*, *A. flavus*, *A. parasiticus*, and *A.* ochraceus. (1) mentioned the inhibitory capability of *Bacillus licheniformis* against the fungi *A. oryzae*, *Colletotrichum sp*., and *A. niger*, with recorded rates of 45%, 40%, and 35%, respectively. (39) explained that the inhibition rate exceeded 80% for the fungus *C. gloeosporioides* when using bacteria. The effectiveness of *Bacillus* bacteria is attributed to changes in cell structure, disruption of energy and transport, and alterations in membrane permeability for fungi (47).

Table 3. Similarity Percentage for the Fungal Isolate *A. flavus* compared to Global Isolates

Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
Transition	264	G/A	ID: OP526902.1	*A. flavus* isolate Afla0001 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,	99%

Table 4. Bacterial Effectiveness in Inhibiting the Growth of *A. flavus*

	Treatment	%Inhibition rate
1	Live bacteria	94.40
2	Killed bacteria	92.36
3	Live bacterial filtrate	88.70
4	Killed bacterial filtrate	72.56
5	Nanoscale bacterial filtrate	89.40
6	Control	0
	L.S.D	0.522

Study of metabolites contained in bacteria

The results show the presence of 98 mg g^{-1} of phenols in live bacteria, 44.8 mg g^{-1} of flavonoids, and 6.9% alkaloids, while saponins recorded 2.11% and glycosides 0.44%, with no tannins detected. The efficiency of live bacteria is attributed to the secondary metabolites they possess. These results align with various studies confirming the efficiency of bacteria, algae, and plant extracts due to their numerous secondary compounds that have the ability to resist pathogenic agents (2). (23) and (14) mentioned the capability of *B. licheniformis* bacteria to produce secondary compounds, including lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, isocoumarins, and glycopeptides (25, 36, 40, 48).

Testing the efficiency of bacteria in preventing the fungus from producing aflatoxin and reducing it: The results in Table (5) demonstrated the bacteria's ability to significantly inhibit aflatoxin production, registering an inhibition rate of 78.309%, compared to the fungus treatment that recorded 0%. The results also showed that the toxin concentration in the isolate producing the toxin was 285.866 ppb, while the isolate producing the toxin with bacteria recorded a concentration of 62.11 ppb.

Table 5. Bacteria Efficiency in Inhibiting *A. flavus* from Aflatoxin B1 Production

	Treatment	Toxin Conc. (ppb)	% Inhibition Rate
1	Toxin-Producing Isolate	285.866	0
2	Fungus + Bacteria	62.11	78.309
	L.S.D	0.0932	0.1008

The results of Table (6) showed the ability of bacteria to reduce the amount of toxin produced from the fungus if the reduction rate reached 85.65%, while it was 0% for treating the fungus alone. The results showed that the concentration of the toxin reached 285.866 ppb in the isolate producing the toxin and 41.08 ppb in treating the fungus with bacteria.

Table 6. Bacteria Efficiency in Reduction *A. flavus* from Aflatoxin B1 production

	Treatment	Toxin Conc. (ppb)	% Reduction ratio
1	Toxin-Producing Isolate	285.866	0
2	Fungus + Bacteria	41.08	85.65
	L.S.D	0.0932	0.0895

The effectiveness of these bacteria is attributed to their ability to produce various inhibitory compounds against microorganisms, mainly consisting of proteins and peptides, most of which are different in structure, such as bacteriocins (42). (23) mentioned that *B. licheniformis* bacteria prevented the synthesis of toxins produced by the fungus *A. flavus*. (44) stated that *B. licheniformis* bacteria have the ability to degrade mycotoxins ZEN, AOH, and AME. Several environmentally safe substances have been used to inhibit toxin-producing fungi and reduce their ability to produce toxins. (10) demonstrated that nano-sea salt at a concentration of 2.5% achieved a 95.8% reduction in toxins, decreasing the toxin concentration from 21.2 parts per billion in the control treatment to 0.89 parts per billion in the nano-salt treatment. (11) found that *Aloe vera* plant extract reduced the production of aflatoxin B1 by 86.5% produced by the fungus *A. flavus* on rice grains.

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