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Combined effects of *Ganoderma lucidum* filtrate and sodium bicarbonate on the growth and Fumonisin B2 producing *Aspergillus niger* isolated from bean grains

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Abstract

Objective

Contamination of food products with mycotoxins represents a serious risk to human health. More than 400 mycotoxins have been reported in food and agriculture products. This research aimed to investigate the combined effects of *G. lucidum* filtrate and sodium bicarbonate on fungal growth and fumonisin B2-producing *A. niger*, providing a potential integrated strategy for controlling toxigenic fungi in stored legumes.

Materials and methods

Bean grain fungus isolates were gathered from retail outlets and local markets in Al-Diwaniyah Governorate, Iraq. Molecular approaches like PCR and morphology were used to isolate and identify fungal species. The macroscopic and microscopic aspects of fungal isolates were initially examined. Genomic DNA from *Aspergillus niger* isolates was extracted. To identify the isolated *Aspergillus niger* strain, the MaximeTM PCR PreMix (i-Taq) Kit was used. The Poisoned Food Technique was used to evaluate the inhibitory effect of *Ganoderma lucidum* filtrate on the radial growth of the toxigenic fungus *Aspergillus niger*. To evaluate the effect of sodium bicarbonate on the radial growth of *Aspergillus niger*, three concentrations of sodium bicarbonate 10, 20, and 30 mg/mL were prepared and incorporated into PDA medium. The combined impact of *G. lucidum* filtrate and sodium bicarbonate on *A. niger's* dry weight by adding three concentrations of each to PDB medium was studied.

Results

Fumonisin B2 was toxic at 112.6 ppb and 8.7 ppb. *A. niger* growth and toxicity were suppressed biologically using *Ganoderma lucidum* filtrate. Fungal growth was suppressed by 69.8% in solid medium and 55.34 percent in liquid media. The chemical treatment of sodium bicarbonate reduced fungal dry biomass by 49.42% in liquid culture and radial growth by 71.4 percent on

solid media. The combination treatment inhibited *A. niger* combined, showing the potential of chemical and biological control techniques.

Conclusion

Overall, the results of this study showed that *Ganoderma lucidum* filtrate and sodium bicarbonate, individually and in combination, are able to inhibit the growth and reduce the biomass of fumonisin B2-producing *Aspergillus niger* in vitro. However, the amount of fumonisin B2 production was not examined after the treatments, and therefore a direct relationship between the inhibition of fungal growth and the reduction of toxin production cannot be confirmed. These findings indicate the potential of these compounds as antifungal agents in vitro and require further studies, including safety assessment, mechanism of action, and application in real food systems.

Keywords: *Aspergillus niger*, fumonisin B2, *Ganoderma lucidum*, HPLC, sodium bicarbonate

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Introduction

Mycotoxin contamination and food deterioration are linked to *Aspergillus niger* (Perrone et al., 2007). Poorly stored agricultural items, particularly bean grains, may be contaminated by the fungus. Despite their nutritional importance, beans may support harmful fungal development. Consequently, contamination of food products with mycotoxins represents a serious risk to human health (Heshmati et al., 2017). More than 400 mycotoxins have been reported in food and agriculture products. Although low level of mycotoxin exposure may not produce obvious clinical signs, continuous consumption of contaminated food at high concentration can result in serious health complication (Shephard, 2008). Contamination of crops and stored products frequently occurs during harvesting, transport, and storage as a result of infection by several fungal genera, especially *Aspergillus, Fusarium, Penicillium*, and *Rhizopus* species (Yimer et al., 2025). The most significant fumonisin produced by certain *A. niger* strains is fumonisin B2 (FB2), a major mycotoxin in food that has been linked to agricultural pollution (Frisvad et al., 2007). Many investigations have found FB2 in *A. niger*-contaminated fruits and cereals (Palumbo et al., 2011; Susca et al., 2016). Fumonisins disrupt sphingolipid metabolism and are hepatotoxic, nephrotoxic, and carcinogenic (Voss et al., 2007). Thus, fumonisin-producing fungi must be identified and monitored to limit food contamination and public health threats. *Ganoderma*

lucidum is bioactive and antifungal (Wasser, 2011; Heleno et al., 2013). Lyousfi et al. (2022) found that sodium bicarbonate may inhibit fungus and spore germination. Highly sensitive and accurate High-Performance Liquid Chromatography (HPLC) is necessary for mycotoxin identification (Kizis et al., 2021). Moreover, biochemical tests are incapable of distinguishing different types of microorganisms, such as bacteria, viruses, and parasites. Genomic techniques such as PCR and sequencing are the most modern practical technology in diagnosing infectious diseases, identifying bacteria, viruses, fungi, and parasites and compared with classical techniques, it has been shown to be more rapid, with results obtained in a few hours, and also more reliable. Genomic techniques allow a faster identification directly from clinical samples. Although the antifungal activities of *Ganoderma lucidum* and sodium bicarbonate have been reported separately, limited information is available regarding their combined application against fumonisin-producing *Aspergillus niger* isolated from bean grains. Therefore, the present study aimed to investigate the combined effects of *G. lucidum* filtrate and sodium bicarbonate on fungal growth and fumonisin B2-producing *A. niger*, providing a potential integrated strategy for controlling toxigenic fungi in stored legumes.

Materials and methods

Isolation and identification of fungi from bean grains: Bean grain fungus isolates were gathered from retail outlets and local markets in Al-Diwaniyah Governorate, Iraq. Fungal contamination in these samples was sought and isolated. Molecular approaches like PCR and morphology were used to isolate and identify fungal species. The macroscopic and microscopic aspects of fungal isolates were initially examined. Morphology was determined by genus and species using well-established taxonomic standards. The research also examined colony morphology (color, texture, form) and microscopic features (sporangia, spores, size, shape, arrangement). Polymerase chain reaction (PCR) investigation confirmed the species' morphological categorization using genetic markers. This approach accurately identified dangerous fungal isolates like *Aspergillus niger*.

Preparation of fungal filtrate: For 15 minutes at 121°C and 15 pressure, glass beakers containing 100 mL of PDB medium sterilized *Aspergillus niger* and *Ganoderma lucidum* fungal filtrates. The contaminated beakers received 250 mg/L chloramphenicol after chilling. Two 7-day-old 5 mm mycelial discs of *A. niger* and *G. lucidum* were inoculated into each flask and incubated at 25 °C for three weeks with daily agitation. Filtrates were kept at 4°C after passing through 0.22µm filters for future use.

Mycotoxin detection by HPLC: *Asperjillus niger* isolates' Fumonisin B2 (FB2) synthesis was assessed using a modified version of Cebadero-Domínguez et al. (2025)'s HPLC approach. The experiment used a Shyam HPLC system with a 25 cm × 4.6 mm C18-ODS column and a fluorescence detector tuned to 335 nm for excitation and 440 nm for emission. The mobile phase was 77:23 (v/v) methanol and 0.1 M NaH2PO4 pH 3.35 with o-phosphoric acid. One milliliter per minute was the mobile phase flow rate. This technique increased FB2 separation and sensitivity detection, allowing exact toxin measurements in samples. Using a standard calibration curve, fungal filtrate fumonisin B2 (FB2) concentration was quantified in ppb.

Molecular Identification of *Aspergillus niger* Using PCR-Extraction and purification of genomic DNA: G-Spin DNA extraction kit instructions were used to extract genomic DNA from *Aspergillus niger* isolates. Fungus mycelia were frozen in liquid nitrogen and lysed using Proteinase K and lysis solution. The lysate was passed over GD columns to bind DNA, washed to remove impurities, then eluted with elution buffer for PCR. (Umesha et al., 2016).

PCR amplification and gel electrophoresis: To identify the isolated *Aspergillus niger* strain, the MaximeTM PCR PreMix (i-Taq) Kit (iNtRoN Biotechnology, South Korea) was used. The reaction mixture (20 µL reaction volume) consisted of 1 µL each of the forward (5′-TGTCTGAAAGCGTGCAGTCT-3′) and reverse (5′TTCAGCGGGTATCCCTACCT-3′) primers, 1 µL of genomic DNA extracted from the *A. niger* isolate, and nuclease-free water to adjust the final volume. All components were added to the PCR tubes pre-loaded with lyophilized reagents from the kit. Amplification was carried out under the following conditions: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation (95 °C, 45 s), primer annealing (58 °C, 30 s), and extension (72 °C, 1 min); followed by a final extension at 72 °C for 7 min. The PCR products were subsequently analyzed by agarose gel electrophoresis to verify the presence and size of the target DNA fragment, confirming successful amplification of the ITS region-specific to *A. niger* (White et al., 1990; Raja et al., 2017).

Effect of *G. lucidum* filtrate on the radial growth of *Aspergillus niger* on PDA medium: The Poisoned Food Technique, described by Dixit et al. (1976), was used to evaluate the inhibitory effect of *Ganoderma lucidum* filtrate on the radial growth of the toxigenic fungus *Aspergillus niger*. Three concentrations of the fungal filtrates 10%, 20%, and 30% were incorporated into sterilized Potato Dextrose Agar (PDA) medium. The medium was thoroughly mixed and poured into sterile Petri dishes and allowed to solidify. A 5 mm mycelial disc was removed from a 7-day-old *A. niger* colony using a sterile cork borer to collect the filtrate. The disk was inserted in the middle of each PDA plate. We performed each therapy three times. The plates were in a 25°C incubator for seven days. Under the same circumstances, control plates were made without *G. lucidum* filtrate. These plates were compared. Following the incubation period, fungal radial growth was measured using a ruler by determining the average diameter of the colonies in two perpendicular directions. Radial growth inhibition was assessed when the fungal growth in the control plates reached the edge of the Petri dish. The percentage of fungal growth was calculated according to Abbott's formula (Miastkowska et al., 2020; Mousa et al., 2021).

Effect of sodium bicarbonate concentrations on the radial growth of *Aspergillus niger*: To evaluate the effect of sodium bicarbonate on the radial growth of *Aspergillus niger*, three concentrations of sodium bicarbonate 10, 20, and 30 mg/mL were prepared and incorporated into PDA medium. The Poisoned Food Technique, outlined by Dixit et al. (1976), was used to evaluate the antifungal activity. The previously described procedures were followed to assess the effectiveness of sodium bicarbonate against *A. niger*.

Effect of the interaction between *Ganodermd lucidum* filtrate and sodium bicarbonate on the radial growth of *Aspergillus niger*: To evaluate the combined effect of *G. lucidum* filtrate and Sodium Bicarbonate on the radial growth of *Aspergillus niger*, three different concentrations of *G. lucidum* (10%, 20%, and 30%, v/v) and sodium bicarbonate (10, 20, and 30 mg/mL) were

prepared. Equal concentrations of both *G. lucidum* filtrate and sodium bicarbonate were incorporated into PDA medium to formulate the combined treatments. The poisoned food technique was utilized to assess the inhibitory interaction between the two agents. A control group comprising untreated PDA plates was included for comparative analysis.

Effect of *Ganoderma lucidum* filtrate on the dry weight of *Aspergillus niger* in PDB: The effect of *G. lucidum* filtrate on the biomass production of *A. niger* was assessed by preparing three concentrations 10%, 20%, and 30% in PDB medium. 50 mL of the treated liquid medium was dispensed into 250 mL sterilized conical flasks. A sterile cork borer was used to extract two 5 mm mycelial discs off the outer edge of a 7-day-old *A. niger* colony and insert them in each flask. The flasks were stored at 25°C for seven days after three treatments. Flabs were mixed twice daily to maintain equal development and aeration throughout incubation. Control flasks with PDB and no filtrate were held under the same conditions. Filtering using pre-weighed filter paper removed fungal biomass after incubation. The fungal mycelium-containing filter sheets were ready to use after 24 hours of drying in a 60°C oven.

Dry Weight (g) = (Weight of dried filter paper with mycelium) − (Weight of empty filter paper)

The method of Pinto et al. (2006) was used to calculate fungal dry weight inhibition % using the following equation.

$$\text{Inhibition percentage (\%)} = \frac{\text{Average of dry weight in control} - \text{Average of dry weight infungus treatment}}{\text{Average of dry weight in control}} \times 100$$

Effect of sodium bicarbonate on the dry weight of the *Aspergillus niger* in PDB: We added 10, 20, and 30 mg/mL sodium bicarbonate to pre-prepared PDB medium to test *Aspergillus niger* biomass generation. The Poisoned Food Technique, developed by Dixit et al. (1976), was used to assess how sodium bicarbonate influenced *A. niger* fungal biomass. After incubation, the dry weight of the fungus was compared to the untreated control flasks to evaluate whether sodium bicarbonate hindered fungal biomass production.

Effect of the interaction Between Sodium Bicarbonate and *Ganoderma lucidum* Filtrate on the Dry Weight of *Aspergillus niger* in PDB: We tested the combined impact of *G. lucidum* filtrate and sodium bicarbonate on *A. niger's* dry weight by adding three concentrations of each to PDB medium. Sterilized 250 mL conical flasks were filled with 50 mL of each treatment. Standard experimental techniques included fungal disc inoculation, seven days of incubation at 25°C, and filtration and drying of fungal biomass. This experiment examined whether sodium bicarbonate and *G. lucidum* filtrate combined inhibited fungus biomass in liquid culture.

Results and discussion

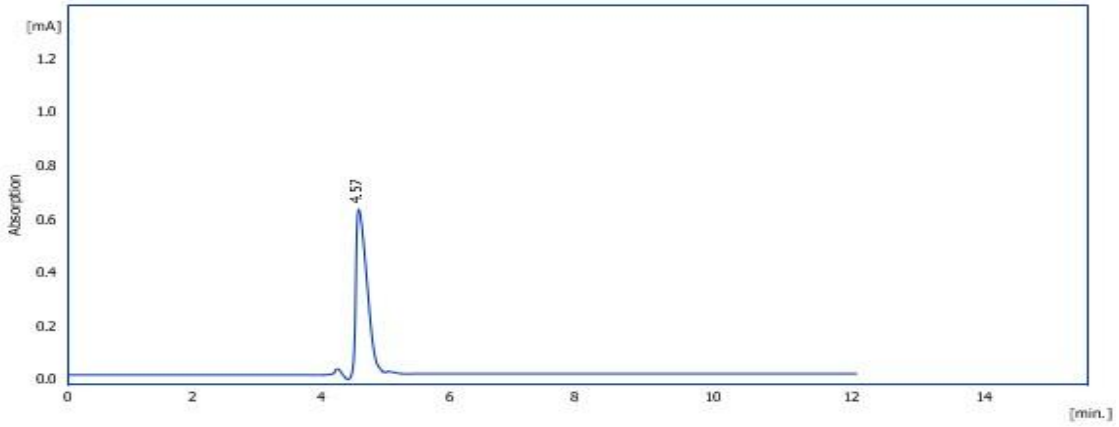
Isolation and identification of fungi: This study involved the isolation and identification of various fungal species linked to bean grains. The identified fungal genera comprised *Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Aspergillus ochraceus, Aspergillus turcosus, Aspergillus sp., Rhizopus stolonifer, Fusarium oxysporum, Penicillium notatum, and Alternaria alternata*. The frequency of fungi occurrence in both surface-sterilized and non-sterilized bean grains samples exhibited significant variation, as presented in Table 1. Surface sterilization was utilized to remove epiphytic fungi, facilitating a more precise differentiation

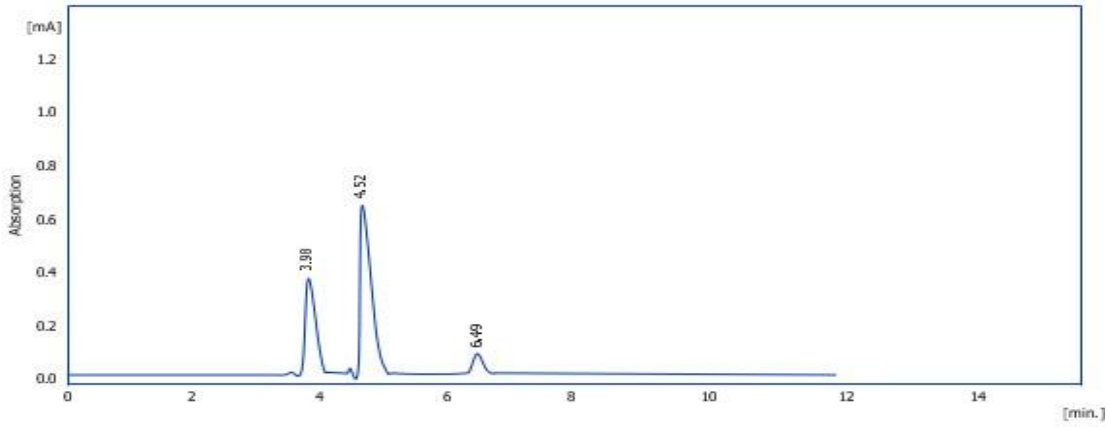
between external and internal colonizers. Among the analysis of isolate fungi, *A. niger* exhibited the most significant prevalence in non-sterilized bean samples, recording an incidence of 93 isolates (58.5%). The frequency in sterilized bean samples reduced to 22 isolates, representing (39.28%) likely due to its high adaptability to storage conditions (Davies et al., 2021). Similarly, *R. stolonifer* recorded 21 isolates (13.2%) in non-sterilized samples and decreased to 17 isolates (30.4%) after sterilization, indicating its close association with surface contamination of grains. These findings agree with previous studies reporting that surface sterilization effectively reduces epiphytic fungal contamination in stored grains (Nečajeva et al., 2023).

Table 1. Fungal species isolated from bean grains

Name of the Isolated Fungus	The percentage of Fungal recurrence in bean grains			
	Superficial sterilized	Superficial non-sterilized	$\chi 2$	P value
Aspergillus niger	22(39.3%)	93(58.5%)	43.835	0.001*
Rhizopus stolonifer	17(30.4%)	21(13.2%)	6.522	0.011*
Penicillium notatum	11(19.6%)	16(10.1%)	2.545	0.111
Aspergillus flavus	2(3.57%)	13(8.2%)	1.690	0.194
Fusarium oxysporum	4(7.1%)	11(7.0%)	3.220	0.073
Aspergillus ochracous	0(0%)	1(0.6%)	0.388	0.533
Aspergillus terreus	0(0%)	1(0.6%)	0.388	0.533
Aspergillus sp.	0(0%)	1(0.6%)	0.388	0.533
Alternaria alternata	0(0%)	1(0.6%)	0.388	0.533
$\chi 2$	25.607	15.390		
P value	0.001*	0.052		

Detection of fumonisin B2 produced by *Aspergillus niger* using HPLC: Fumonisin B2 (FB2), a mycotoxin produced by *A. niger*, was identified through High-Performance Liquid Chromatography (HPLC), as illustrated in Figure 1. Fungal isolates from damaged bean seeds may generate FB2. A natural Fumonisin B2 solution at 10 ppb was utilized for HPLC analysis. *A. niger* isolates from damaged bean grains produced FB2 at quantities ranging from 8.7 ppb to 112.6 ppb. Frisvad et al. (2007) found that *A. niger* isolates may manufacture fumonisin in a controlled setting. This experiment's 4.57-minute FB2 retention duration validated the chemical's identification. The tested isolate's chromatographic study revealed a strong chromatographic peak with a retention time of 4.57 minutes at 1542.98 mAU and 594.74 mAU. However, further tiny peaks at 3.88 and 6.40 minutes of retention had lower peak intensities. Pollutants or fungal secondary metabolites may induce this. Minor peaks may represent secondary metabolites from *A. niger* (Nielsen et al., 2009). HPLC was sensitive and reliable in identifying and quantifying FB2 in *A. niger*-infected bean grain samples.





Result chromatography Table ([illegible]) - F:\ sample [illegible]

No	[illegible]. Time [min]	Area [illegible]	Height [illegible]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	3.98	9124.04	373.50	35.00	35.00	0.15	
2	4.52	17998.74	625.98	60.00	60.00	0.25	
3	6.49	621.40	57.44	5.00	5.00	0.01	
	Total	27744.18	1052.65	100.00	100.00		

Figure 1. Standard results for detection of Fumonisin B2 and sample highest toxin by HPLC technology

DNA sequencing results: ITS-rRNA gene sequencing was used to molecularly define local *Aspergillus niger* isolates (White et al., 1990). ITS-rRNA amplification from *Aspergillus niger* isolates yielded 403 bp PCR fragment (Figure 2). Compare the isolates' sequences to NCBI-BLAST database reference sequences to establish their genetic identity. Tamura et al. (2013) utilized MEGA 6.0 for phylogenetic analysis. We calculated evolutionary distances using Maximum Composite Likelihood and built the phylogenetic tree using Unweighted Pair Group with Arithmetic Mean. According to Figure 3, the local *A. niger* isolate (IQ.BS) shared a lot of genetic material with NCBI reference strains, notably PZ151307.1. Figure 3 shows the numerous sequence alignment findings. Asterisks (*) indicate nucleotide similarity, and local isolate alterations are noted. The additional phylogenetic research confirmed the local Aspergillus IQ isolate (Figure 4).BS and *A. niger* strain MT682456.1 exhibited a significant sequence similarity. The local isolate exhibited 99.95% sequence similarity with comparable Aspergillus strains. High sequence identity confirms that the local isolate is *A. niger* and that the ITSrRNA region is valid

for molecular identification. Through this analysis, the local isolate was confidently identified as *A. niger*. The ITS sequence of the *Aspergillus* IQ.BS isolate has been deposited in GenBank with the accession number PZ151307.1.

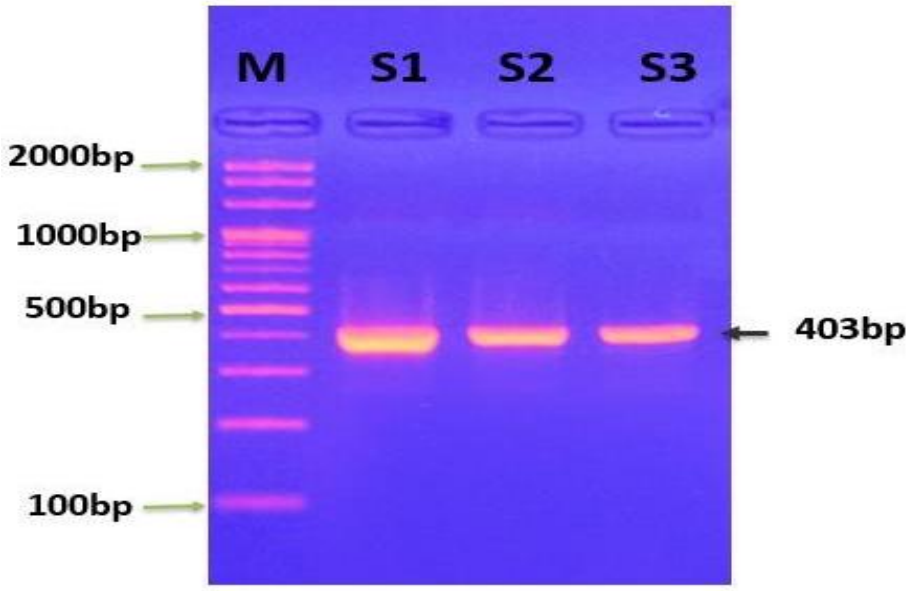


Figure 2. Agarose gel electrophoresis of PCR-amplified ITS-rRNA gene fragments from *Aspergillus niger* isolates. Lane M represents the DNA marker (100–2000 bp), while lanes S1–S3 showed positive amplification bands at approximately 403 bp corresponding to the ITS region of *A. niger*

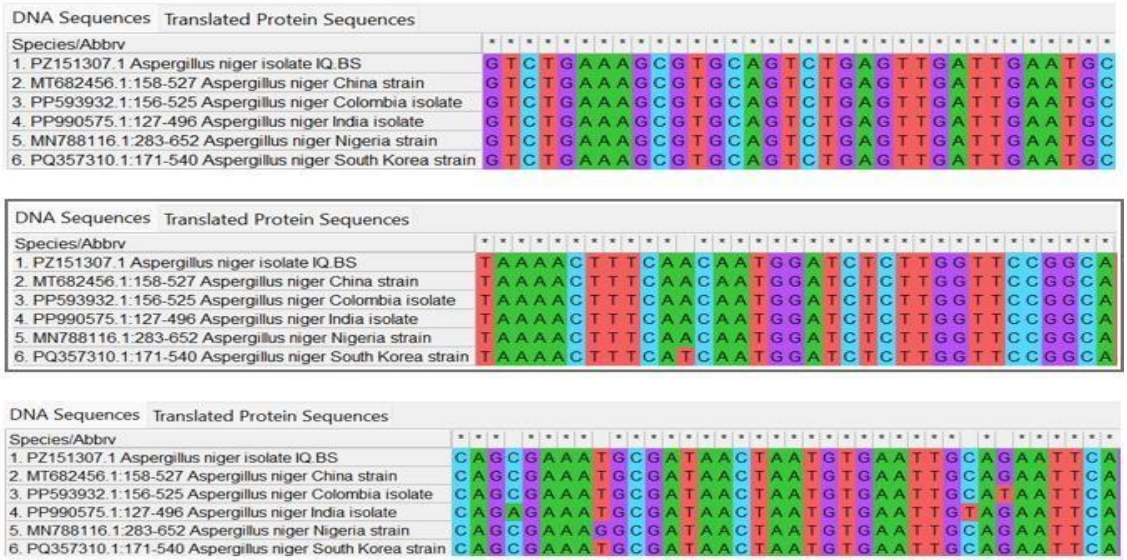


Figure 3. Multiple sequence alignment analysis of Internal transcribed spacer ribosomal RNA gene in local *Aspergillus niger* bean grains isolate and NCBI-GenBank fungi related *Aspergillus niger* strains. The multiple alignment analysis was constructed using (ClustalW alignment tool. Online). That alignment analysis showed the nucleotide alignment similarity as (*) and substitution mutations in Internal transcribed spacer ribosomal RNA gene between isolates

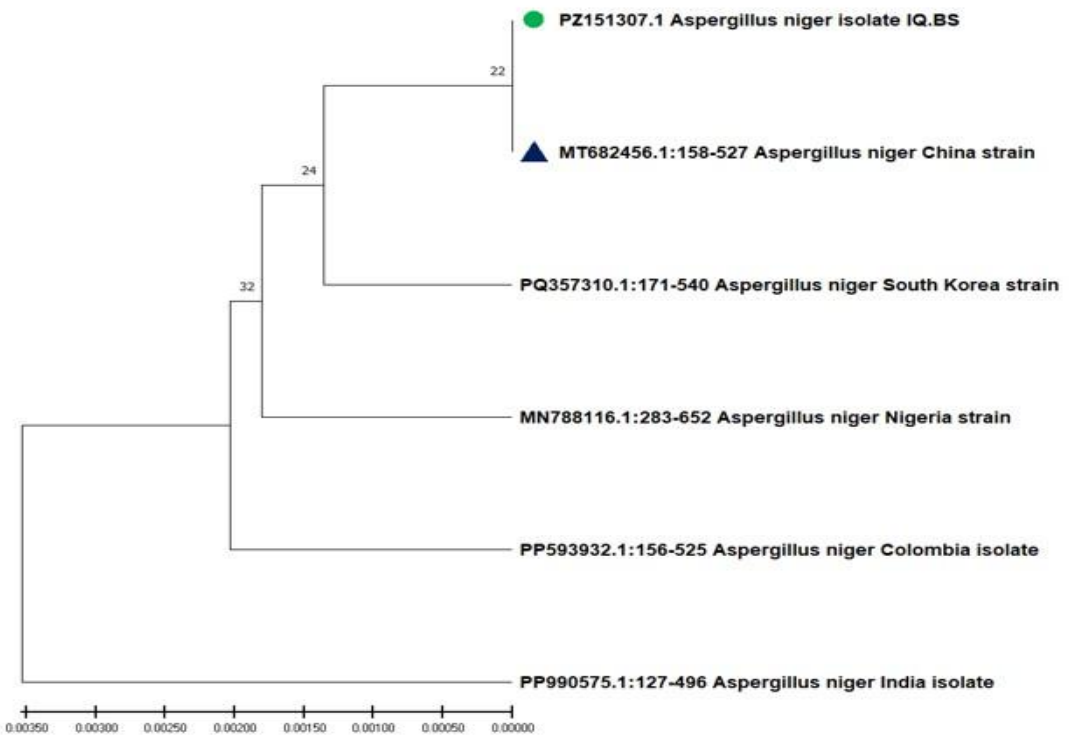


Figure 4. Phylogenetic tree analysis based on partial ITS1 rRNA gene sequences of the local *Aspergillus niger* IQ.BS isolate used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree method) and the evolutionary distances were computed using the Maximum Composite Likelihood method (MEGA 6.0 version). The local *Aspergillus niger* IQ.BS isolate showed high genetic similarity with the reference *Aspergillus niger* strains retrieved from the NCBI-BLAST database, particularly the China strain, with low evolutionary divergence values ranging from 0.0020 to 0.006

Effect of *Ganoderma lucidum* Filtrate on the Radial Growth of *Aspergillus niger* on PDA: The results presented in Table 2 demonstrated the application of *G. lucidum* filtrate at different concentrations had a significant effect on the radial growth of *A. niger*. The maximum inhibition rate recorded was 69.8% in plates with 30% *G. lucidum* filtrate, resulting in an average colony diameter of 2.56 cm, in contrast to the control treatment, which exhibited an average diameter of 8.5 cm. At a concentration of 10%, the mean colony diameter measured 5.0 cm, which correlated to a 41.8 % inhibition rate. As concentration increased to 20%, colony diameter decreased to 3.06 cm and inhibition rate increased to 60.6%. Results showed a clear link between *G. lucidum* filtrate concentration and *A. niger* radial growth inhibition. The inhibitory impact was consistent with the results of Meena et al. (2017) and Vilela et al. (2025). Given the filtrate's high concentration of antibacterial agents, antioxidants, and physiologically active substances, these molecules may help inhibit fungus development. (Heleno et al., 2013).

Table 2. Effect of *G. lucidum* filtrate, sodium bicarbonate and their combined treatment on the radial growth of *A. niger*

Concentration mg/mL percentage and dilutions	*G. lucidum* treatment (%)		Sodium bicarbonate treatment (mg/mL)		Combined treatment *G. lucidum* + Sodium bicarbonate		Mean ± SD
	Diameter (cm)	Inhibition (%)	Diameter (cm)	Inhibition (%)	Diameter (cm)	Inhibition (%)	
10	5.0±1.0	41.8	4.5±0.62	45.6	4.27±0.68	51.0	4.59±0.37
20	3.06±0.14	60.6	2.93±0.23	64.4	3.03±0.45	65.6	3.00±0.26
30	2.56±0.41	69.8	2.24±0.25	71.4	2.3±0.50	73.2	2.37±0.17
Control	8.5		8.5		8.5		
Mean ± SD	3.54±1.23		3.22±		3.20±0.98		
LSD	1.73		1.49		1.12		
P-value	0.007		0.001*		0.013		

Effect of sodium bicarbonate on the radial growth of *Aspergillus niger* on PDA: Table 2 shows that sodium bicarbonate limits *A. niger* radial development on PDA media. All dosages of sodium bicarbonate inhibited fungal growth relative to the control group. At 10 mg/mL, *A. niger* colonies averaged 4.5 cm, resulting in a 45.6% inhibition rate. With 20 mg/mL dosage, colony diameter decreased to 2.93 cm and inhibition rate increased to 64.4%. Suppression was greatest at 30 mg/mL, with a 2.24 cm colony diameter and 71.4% inhibition. The control treatment without sodium bicarbonate had an average colony diameter of 8.5 cm. Alkalinity makes sodium bicarbonate antifungal by raising pH and making fungus unfriendly. Fernandes et al. (2017) found that salt compounds inhibited dangerous fungi similarly.

Effect of the interaction between *Ganoderma lucidum* filtrate and sodium bicarbonate on the radial growth of *Aspergillus niger* on PDA: Table 2. shows that *G. lucidum* filtrate and sodium bicarbonate affected *A. niger's* radial growth on PDA medium. The combination of these two drugs at various doses greatly inhibited fungal growth. Compared to each medicine alone, antifungal filtrate and sodium bicarbonate had greater inhibitory effects. A mean colony diameter of 2.3 cm at 30% dose showed 73.2% inhibition. At 20% concentration, the colony diameter was 3.02 cm with 65.6% inhibition. The 10% concentration yielded a 51% inhibition rate with an average colony diameter of 4.27 cm. Due to its combined effects, the combination therapy surpassed the control treatment, which had an average colony diameter of 8.5 cm without inhibition. *G. lucidum* filtrate and sodium bicarbonate combination dosages promote fungal growth inhibition, demonstrating concentration-dependent antifungal efficacy.

Effect of *Ganoderma lucidum* filtrate on the dry weight of *Aspergillus niger* in PDB: *G. lucidum* filtrate significantly reduced *A. niger* biomass production in PDB medium (Table 3). Concentration-dependent inhibition reduced fungal dry weight less at higher filtrate

concentrations. At 30% filtrate concentration, *A. niger'* mean dry weight decreased to 0.461 g, the inhibition rate was 55.34 percent. At a concentration of 20% the fungal dry weight decreased to 0.545 g corresponding to an inhibition rate of 47.18%. At the minimum concentration evaluated (10%), the dry weight measured 0.629g, exhibiting an inhibition rate of 39.10%. The results suggested that the inhibitory effect of *G. lucidum* filtrate on fungal biomass increased proportionally with concentration in liquid culture. The inhibitory activity observed in the present study may be associated with bioactive metabolites previously reported in *G. lucidum* (Thapa et al., 2025); however, the composition of the filtrate was not characterized in the current investigation. These findings are consistent with the observations reported by Hussien and Saadon (2022), supporting the antifungal potential of *G. lucidum*, which may be attributed to its bioactive metabolites capable of suppressing fungal growth in vitro.

Table 3. Effect of *G. lucidum* filtrate, sodium bicarbonate and their combined treatment on the dry weight and inhibition percentage of *A.niger*

Concentration mg/mL and percentage of dilutions	Treatments *G. lucidum* (%)		Sodium bicarbonate treatments (mg/mL)		Combined treatments *G. lucidum* + sodium bicarbonate		Mean ± SD
	Weight (g)	Inhibition (%)	Weight (g)	Inhibition (%)	Weight (g)	Inhibition (%)	
10	0.629±0.022	39.10	0.632±0.057	38.80	0.426±0.014	58.69	0.562±0.114
20	0.545±0.035	47.18	0.568±0.018	44.99	0.346±0.031	68.43	0.480±0.134
30	0.461±0.028	55.34	0.522±0.012	49.42	0.233±0.008	78.12	0.403±0.157
Control	1.0324		1.0324		1.0324		
Mean ±SD	0.545±0.084		0.574±0.055		0.326±0.100		
LSD	0.047		0.047		0.047		
P-value	0.001*		0.001*		0.001*		

Effect of sodium bicarbonate on the dry weight of *Aspergillus niger* in PDB medium: The results presented in Table 3 demonstrated that sodium bicarbonate exhibited a significant inhibitory effect on the biomass (dry weight) of *A. niger* in Potato Dextrose Broth (PDB) medium, with inhibitory rates varying according to concentration. An inhibition rate of (49.42%) was observed at a concentration of 30 mg/mL, resulting in an average dry weight of the fungal biomass being reduced to 0.522 g. The average dry weight at a concentration of 20 mg/mL was measured at 0.568g, which corresponds to an inhibition rate of (44.99%). The minimum inhibition rate recorded was (38.80%) at the concentration of 10 mg/mL, corresponding to an average dry weight of 0.632 g. A concentration-dependent inhibitory effect of sodium bicarbonate was observed, with antifungal activity increasing at higher concentrations. These findings are consistent with those reported by Guo et al. (2023), who demonstrated that increasing sodium bicarbonate concentration significantly reduced fungal biomass.

Effect of the interaction between sodium bicarbonate and *Ganoderma lucidum* filtrate on the dry weight of *Aspergillus niger* in PDB: The combined application of *G. lucidum* filtrate

and sodium bicarbonate showed a more significant inhibitory effect on the dry weight of *A. niger* compared to each treatment applied individually. The data presented in Table 3 indicate that the peak inhibition rate of 78.12% occurred at a concentration of 30%, reducing the fungal biomass dry wight to 0.233 g. The dry weight at a concentration of 20% was measured at 0.346 g, which indicated an inhibition rate of 68.43%. At the lowest dose (10%), the fungus had 0.426 g dry weight and 58.69% inhibition. The control treatment, which did not include antifungal agents, exhibited an average dry weight of 1.0324 g. These results indicated a combined effect between *G. lucidum* filtrate and sodium bicarbonate in inhibiting fungal biomass production. The combined effect of the treatments surpassed the efficacy of each agent used independently, indicating that the bioactive compounds in *G. lucidum* when combined with the alkalizing properties of sodium bicarbonate, significantly enhanced antifungal activity in liquid culture systems (Thapa et al., 2025). The present study has some limitations. Although significant antifungal activity was observed, the specific bioactive compounds responsible for the inhibitory effect of *Ganoderma lucidum* filtrate were not identified. In addition, metabolomic profiling and mechanistic investigations were beyond the scope of this study. Future research should focus on the characterization of antifungal metabolites, elucidation of their mode of action, and evaluation of their effects on fumonisin biosynthesis pathways. Another limitation of the present study is that fumonisin B2 production was assessed only for the identification of the toxigenic potential of the *Aspergillus niger* isolate. The effect of *Ganoderma lucidum* filtrate and sodium bicarbonate treatments on FB2 biosynthesis was not evaluated. Since fungal growth inhibition does not necessarily result in a proportional reduction in mycotoxin production, further studies are required to quantify FB2 levels after treatment and to investigate the influence of these agents on toxin biosynthesis pathways. The third limitation of the current study is the absence of biochemical characterization of the *Ganoderma lucidum* filtrate. Parameters such as pH, protein content, polysaccharide concentration, total phenolic content, and metabolite composition were not determined. Consequently, the specific compounds responsible for the observed antifungal activity could not be identified. Future studies should include detailed chemical and metabolomic analyses to establish relationships between filtrate composition and antifungal efficacy.

Conclusion: *Aspergillus niger* was isolated from bean seeds and identified and confirmed using PCR and DNA sequencing. This isolate was able to produce fumonisin B2 up to a level of 112.6 ppb, which was confirmed using HPLC. *Ganoderma lucidum* filtrates inhibited fungal growth by 69.8% in PDA medium and reduced biomass by 55.34% in PDB medium. Sodium bicarbonate also reduced growth by 71.4% and biomass by 49.42%, respectively. In the combination treatment, the growth inhibition rate increased to 73.2% and biomass reduction increased to 78.12%, indicating an enhanced inhibitory effect in vitro, without this effect being considered as a confirmed synergy based on standard tests. Overall, this study demonstrates the in vitro antifungal potential of *G. lucidum* filtrates and sodium bicarbonate against fumonisin B2-producing *A. niger*. However, post-treatment fumonisin B2 levels, MIC/MFC assays, standard positive controls, and safety, toxicity, sensory, and residue studies were not performed in this study. Therefore, any potential applications in food systems are purely hypothetical and require further studies in real-world conditions, food models, and in vivo.

Author contributions

N. K. F. conceived and designed the study, performed the experimental work, collected the samples, conducted laboratory analyses, analyzed the data, and prepared the original draft of the manuscript. A. A. S. supervised the research, contributed to the study design and interpretation of results, reviewed and edited the manuscript, and approved the final version for publication. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical considerations

This study did not involve human participants or experimental animals. All laboratory procedures were conducted in accordance with the institutional regulations and biosafety guidelines of the Department of Biology, College of Science, University of Al-Qadisiyah, Iraq.

Conflict of interest

The authors declare no conflict of interest.

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اثرات ترکیبی فیلتراسیون *Ganoderma lucidum* و بی‌کربنات سدیم بر رشد و تولیدکننده فومونیزین B2 جداشده از دانه‌های لوبیا *Aspergillus niger*

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چکیده

هدف: آلودگی مواد غذایی به مایکوتوکسین‌ها یکی از خطرات جدی برای سلامت انسان محسوب می‌شود. تاکنون بیش از ۴۰۰ نوع مایکوتوکسین در محصولات غذایی و کشاورزی گزارش شده است. هدف این پژوهش بررسی اثرات ترکیبی فیلتراسیون قارچ *Ganoderma lucidum* و بی‌کربنات سدیم بر رشد قارچ *Aspergillus niger* تولیدکننده فومونیزین B2 بود تا راهبردی تلفیقی برای کنترل قارچ‌های توکسین‌زا در حبوبات انبارشده ارائه شود.

مواد و روش‌ها: جدایه‌های قارچی از دانه‌های لوبیا جمع‌آوری‌شده از فروشگاه‌ها و بازارهای محلی استان دیوانیه عراق به دست آمدند. شناسایی قارچ‌ها با استفاده از ویژگی‌های ریخت‌شناسی و روش‌های مولکولی از جمله PCR انجام شد. در ابتدا خصوصیات ماکروسکوپی و میکروسکوپی جدایه‌ها بررسی گردید. DNA ژنومی از جدایه‌های *Aspergillus niger* استخراج شد و برای تأیید شناسایی این گونه از کیت Maxime™ PCR PreMix (i-Taq) استفاده شد. اثر بازدارندگی فیلتراسیون *Ganoderma lucidum* بر رشد شعاعی قارچ توکسین‌زای *A. niger* با استفاده از روش غذای مسموم (Poisoned Food Technique) ارزیابی گردید. برای بررسی اثر بی‌کربنات سدیم، سه غلظت ۱۰، ۲۰ و ۳۰ میلی‌گرم بر میلی‌لیتر تهیه و به محیط کشت PDA افزوده شد. همچنین اثر ترکیبی فیلتراسیون *G. lucidum* و بی‌کربنات سدیم بر وزن خشک *A. niger* با افزودن سه غلظت از هر یک به محیط کشت مایع PDB مورد مطالعه قرار گرفت.

نتایج: مقادیر فومونیزین B2 در نمونه‌های مورد بررسی به‌ترتیب ۱۱۲/۶ و ۸/۷ قسمت در میلیارد (ppb) گزارش شد. رشد و سمیت *A. niger* به‌صورت زیستی توسط فیلتراسیون *Ganoderma lucidum* مهار گردید. این تیمار باعث کاهش ۶۹/۸ درصدی رشد قارچ در محیط جامد و ۵۵/۳۴ درصدی در محیط مایع شد. تیمار شیمیایی با بی‌کربنات سدیم نیز موجب کاهش ۴۹/۴۲ درصدی زیست‌توده خشک قارچ در محیط مایع و ۷۱/۴ درصدی رشد شعاعی در محیط جامد گردید. تیمار ترکیبی هر دو عامل بیشترین مهار را بر *A. niger* نشان داد و پتانسیل استفاده همزمان از روش‌های کنترل زیستی و شیمیایی را آشکار ساخت.

نتیجه‌گیری: نتایج این مطالعه نشان داد که فیلتراسیون *Ganoderma lucidum* و بی‌کربنات سدیم، چه به‌صورت منفرد و چه به‌صورت ترکیبی، قادر به مهار رشد و کاهش زیست‌توده *Aspergillus niger* تولیدکننده فومونیزین B2 در شرایط آزمایشگاهی هستند. با این حال، میزان تولید فومونیزین B2 پس از اعمال تیمارها اندازه‌گیری نشد؛ بنابراین نمی‌توان ارتباط مستقیمی بین مهار رشد قارچ و کاهش تولید سم را تأیید کرد. این یافته‌ها پتانسیل این ترکیبات را به‌عنوان عوامل ضدقارچی در شرایط آزمایشگاهی نشان می‌دهد، اما انجام مطالعات تکمیلی شامل ارزیابی ایمنی، بررسی مکانیسم اثر و آزمون در سامانه‌های واقعی غذایی ضروری است.

کلمات کلیدی: بی‌کربنات سدیم، فومونیزین B2، کروماتوگرافی مایع با کارایی بالا (HPLC)، *Aspergillus niger*، *Ganoderma lucidum*

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