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Microbial Strategies for Mycotoxin Reduction: Endophytic Fungi and Yeast in Aflatoxin B1 and Citrinin Biocontrol

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ABSTRACT

The ubiquitous threat of Aflatoxin B1 (AFB1) and Citrinin (CIT), potent mycotoxins derived from *Aspergillus* and *Penicillium* species, necessitates innovative strategies for food safety. This study aimed to assess fungal contamination in commercially available, slightly damaged canned food products from Egyptian markets. Five *Aspergillus* species were isolated and tested for their AFB1 and CIT production capabilities. The study then investigated the potential of endophytic fungi isolated from *Solanum nigrum* and *Urtica dioica* to mitigate these mycotoxins via their metabolites and binding activities. *Alternaria tenuissima* (accession number PQ517551) emerged as the most effective endophytic fungal species for mycotoxin reduction; its purified extract (band 2) achieved a 90.17% reduction in CIT and a 21.09% reduction in AFB1. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of this extract revealed 12 bioactive metabolites, including five fatty acids with known anti-mycotoxigenic properties. Concurrently, *Meyerozyma guilliermondii* (accession number PV660158), the second most effective endophytic isolate, exhibited potent AFB1 binding, achieving reduction rates ranging from 48.56% to 87.42% under influence of cell viability, incubation period and pH of the medium. These findings highlight the potential of specific endophytic fungal strains as effective strategies for mitigating AFB1 and CIT contamination.

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi, posing significant threats to public health (Zahra *et al.*, 2019). The presence of mycotoxins in food and feed poses a significant threat to their quality, safety, and consumer health (Shekhar *et al.,* 2025). They have been found to exert a wide range of detrimental effects (Dai *et al.,* 2022; Sun *et al.,* 2022). Researchers have dedicated substantial efforts to mitigating the harmful effects of mycotoxins, exploring physical, chemical, and biological methods for their removal, detoxification, and degradation. Nevertheless, the widespread application of these methods is hindered by safety concerns and other challenges (Li *et al.,* 2025).

A lot of attention is being directed toward the biological detoxification of mycotoxins as it offers a safer, more environmentally friendly and effective solution compared to chemical and physical methods, which often have high costs and negative impacts on food safety and nutritional value (Ndiaye *et al.,* 2022; Zhu *et al.,* 2017). Recent studies have increasingly focused on microbiological approaches for mycotoxin decontamination (Assaf *et al.,* 2019; Ismaiel *et al.,* 2023; Fovo *et al.,* 2025).

Aflatoxins (AFs) are among the most potent mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The consumption of agricultural commodities contaminated with AFs poses substantial public health risks, evidenced by adverse health consequences in human and animal populations, including fatal outcomes in severe cases(Okechukwu *et al.,* 2024). Several types of AFs exist, but aflatoxin B1 (AFB1) is recognized as the most toxic and carcinogenic type (Huang *et al.*, 2019).Various microorganisms, including fungi, bacteria and yeasts, show a reduction of AFs (Ren *et al.,* 2020). The co-cultivation of *A. flavus* and *A. parasiticus* with *Salmonella* led to a decrease in the diameter of colonies and spore formation for both fungal species, and the levels of AFs were reduced (von Hertwig *et al.*, 2020). Inhibitory substances released by a non-aflatoxigenic *A. flavus* and *A. oryzae* can suppress AFB1 synthesis as well as the growth and reproduction of aflatoxigenic *A. flavus* (Yang *et al.*, 2020).

Citrinin (CIT) was firstly discovered in 1930s by Hetherington and Raistrick from a *Penicillium citrinum* culture (He and Cox, 2016). The genera *Aspergillus, Penicillium*, and *Monascus* are the main producers of CIT (Ostry *et al.,* 2013; He and Cox, 2016). It has been discovered in food colorants, often produced in Asia through the fermentation of rice with *Monascus purpureus* (red mold rice). Besides its use as a food coloring agent, it is also sold in Europe as a dietary supplement for lowering cholesterol levels (Farawahida *et al.,* 2022). Although CIT exhibits a diverse range of bioactivities *in vitro*, including antibacterial, antifungal, potential anticancer, and neuroprotective characteristics, it is seldom used as a drug due to its observed harmful effects on mammals, including nephrotoxicity, teratogenicity, carcinogenicity, embryotoxicity, and also induced nephropathy in humans (Ali *et al.,* 2015; Zargar and Wani, 2023). In recent years, many studies have documented the biological degradation of CIT. *Trichoderma hamatum* was used to minimize CIT generated by *Penicillium viridicatum* in rice grains (Abd-Allah and Ezzat, 2005). *Rhizobium borbori* PS45 has shown to be capable of degrading CIT grown on mineral media (Kanpiengjai *et al.,* 2016). Furthermore, *Rhodotorula mucilaginosa* achieved a 91.67% *in vitro* degradation of CIT from *Penicillium digitatum* (Ahima *et al.*, 2019).

Recently, treatments involving yeasts and yeast derivatives for detoxifying mycotoxins have been suggested. The yeast cell walls, which contain proteins, lipids, and polysaccharides (mannans and glucans), provide a wide variety of readily accessible adsorption sites (Joannis-Cassan *et al.,* 2011). Several studies have demonstrated that mycotoxins are eliminated by adhesion to cell wall components rather than through metabolic degradation or covalent binding, as non-viable cells maintain their capacity to adhere (Sampaio Baptista *et al.*, 2004; Santin *et al.*, 2003). Adsorption capacity is highly dependent on yeast and mycotoxin composition; however, the lack of a clear correlation between yeast composition and adsorption efficiency highlights the complex nature of mycotoxin binding to yeasts (Bzducha-Wróbel *et al.,* 2019).

This study aimed to investigate the capacity of endophytic fungi to biologically mitigate AFB1 and CIT, produced by

fungal contaminants of canned foods, via their metabolites and binding properties.

MATERIALS AND METHODS

Canned Food Samples:

Seven different types of unexpired canned food products were purchased from different Egyptian markets includes tuna (3 samples), smoked tuna (1 sample), Anchovy (1 sample), Mackerel (2 samples), Salmon (1 sample), Sardine (2 samples) and Bully beef (2 samples). The cans were of good quality, with only slight external damage. They were transported to the laboratory and analyzed immediately for fungal contamination.

Collection of Plant Samples:

Solanum nigrum and *Urtica dioica* are two medicinal plants collected from the gardens of Cairo University, Egypt. Healthy plant samples were packed in sterile polyethylene bags, transported to the laboratory, and immediately used for the isolation of endophytic fungi.

Detection of Fungal Contamination in Canned Foods:

Direct plating technique was used to evaluate the mycological quality of canned food samples. The procedure was carried out as explained by Baiyewu *et al.* (2007) with minor modifications. An approximately 3 cm^3 segments of tissues of each canned food products were cut using a sterile scalpel and forceps, then three of these segments transferred onto Petri plates containing Czapek Yeast Autolysate Agar (CYA) medium supplemented with chloramphenicol (50 µg/mL) to inhibit bacterial proliferation then incubated at 28°C for 7 to 10 days. Pure isolates were transferred to slants and kept at 4°C for further studies.

Isolation of Endophytic Fungi from *S. nigrum* and *U. dioica*:

According to ALKahtani *et al.* (2020), endophytic fungal species were isolated from stems and leaves of *S. nigrum* and *U. dioica*. Collected leaves and stems were rinsed with tap water and then prepared for surface sterilization. This preparation involved uniformly cutting segments, including the midrib, from their mid-portions using sterile surgical blades. Then, the segments were immersed in sterile distilled water for 1 min, 70% ethyl alcohol for 1 min, 2.5% sodium hypochlorite for 4 min, and 70% ethyl alcohol for 30 sec. Finally, the segments were subjected to a triple rinse in sterile distilled water. Excess water was removed by air-drying segments on sterile Whatman no. 1 filter paper. Five segments were placed onto the surface of petri plates containing CYA agar medium supplemented with chloramphenicol (50 µg/mL) to inhibit bacterial proliferation then incubated at 28°C until the outgrowth of endophytic fungi were observed. Each developed fungal isolate was sub-cultured into a fresh medium for 1 week at 28°C, then transferred to slants and kept at 4°C for further studies.

Morphological Identification of Isolated Fungi:

Fungal isolates were morphologically identified by examining colony characteristics (color, texture) and microscopic features (fruiting bodies, spore morphology), using the identification keys provided by and Gilman (1957) and Moubasher (1993).

Inoculum Preparation:

All isolates were induced to sporulate on slants at 28 °C for 7 days. The spores of each isolate were collected by covering the slants with sterile distilled water containing 0.1% Tween 20, then carefully scraping the spores using a sterile glass rod. Spore suspensions were then adjusted to 10^6 spores/mL with hemocytometer.

Screening of Fungi Isolated from Canned Foods for AFB1 and CIT Production:

One mL of the spore suspension of each fungal isolate was inoculated into 250 mL Erlenmeyer flask containing 100 mL of CYA broth medium. Triplicate culture flasks for each fungal isolate were incubated in shaking incubator at 150 rpm for 10 days in the dark at 28°C. After

incubation, the cultures were filtered through Whatman No. 1 filter paper. For AFB1 and CIT detection, the filtrate of each flask was extracted with an equal volume of chloroform three successive times, then the mixture was shaken for 30 min and left in the separating funnel until the formation of two separate layers. The chloroform phase was filtered over anhydrous sodium sulfate and subsequently evaporated to dryness under vacuum using rotary evaporator. After being dried, the crude extract was resuspended in absolute methanol to be analyzed by thin layer chromatography (TLC) (Ismaiel *et al.,* 2022).

Determination of AFB1 and CIT Using TLC:

TLC plates were prepared following the method described by Stahl (1969). A slurry was formed by mixing 10 g of silica powder (GF-254) with 30 ml of warm distilled water. After that, the mixture was transferred to a glass plate (20×20 cm) and left to solidify at room temperature. The plates were used once cooled, following a 1-hour heating period in an electric oven at 110°C. The dried crude fungal extracts that resuspended in absolute methanol (200 µL), were spotted on TLC plates along with AFB1 and CIT standard solutions (Sigma Aldrich, Taufkirchen, Germany), which were subsequently developed in toluene: ethyl acetate: formic acid (60:30:10, v/v) solvent system. Once the solvent system reached the end of the plates by 2 cm, plates were allowed to dry at room temperature, then UV light was used to visualize the bands of samples and standards as blue fluorescence for AFB1 and fluorescent lemon-yellow for CIT at short (254 nm) and long (366 nm) wavelengths. The rate of flow (Rf) of AFB1 and CIT is determined using the following formula (Snyder, 2008).
Rf = Distance travelled by substance / Distance travelled by the solvent front.

The bands of standards and samples scrapped off from TLC plate, eluted in methanol for centrifugation. The supernatants of AFB1 and CIT were determined using a UV spectrophotometer (6800 UV/Vis. Spectrophotometer, JENWAY) at 363 nm and 366 nm, respectively, with methanol as the control. Concentrations were determined using standard curve (El-Shanawany *et al.,* 2005; Ismaiel and Tharwat, 2014).

Effect of the Endophytic Fungi on AFB1 and CIT Production:

To determine the effect of the endophytic fungi on the production of AFB1 and CIT, procedure according to Ahima *et al.* (2019) was employed with minor modifications. Interactive mixed cultures of each of the AFB1 and CIT producing fungi, along with each of the tested endophytic fungal strains were co-inoculated in 50 ml of CYA broth medium with 1 ml of each spore suspension (10^6 spores/mL). Flasks singly inoculated with the AFB1 and CIT producing fungi were used as controls. All flasks were incubated in the dark at 28°C for 10 days. After incubation, the cultures were filtered through Whatman No. 1 filter paper. AFB1 and CIT residues were extracted and quantified spectrophotometrically as previously mentioned. The percentage of reduction of mycotoxins was calculated using the following equation:

$$\% \text{ Reduction} = (C_0 - C / C_0) \times 100$$

where C_0 and C represented the concentrations of the mycotoxins in the control and treated samples, respectively.

Molecular Identification of the Selected Fungal Isolates:

The molecular characterization of the most potent endophytic fungal isolates, as well as fungi that produce CIT and AFB1, was conducted via sequencing of the polymerase chain reaction (PCR) amplified internal transcribed spacer (ITS) region at the Animal Health Research Institute, Egypt. Fungal DNA was extracted using QIAamp DNeasy Plant Mini kit instructions. Ribosomal RNA gene (18S rDNA) amplification was achieved through PCR employing universal primers ITS1 and ITS4, with nucleotide sequences 5' – TCC GTA GGT GAA CCT GCG G - 3' and 5'-TCC TCC GCT TAT TGA TAT GC-3',

respectively. The thermal cycling parameters used were initial denaturation at 94 ˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 56˚C for 40 sec, and extension at 72˚C for 50 sec, with a final extension at 72˚C for 10 min. The PCR products were purified using the QIAquick PCR purification kit (Qiagen Inc.Valencia CA) according to the manufacturer's protocol. The purified PCR products were sequenced in the forward and reverse directions using Sanger sequencing on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Resultant nucleotide sequences of the identified fungi were deposited in GenBank, and their accession numbers were obtained. They were then compared with those available in the public online databases of the NCBI (National Center for Biotechnology Information) using the BLAST (Basic Local Alignment Search Tool) search program (http://www.ncbi.nlm.nih.gov/blast/) (Altschul *et al.,* 1990). Phylogenetic analysis was performed using the Neighbor-Joining method in MEGA 12 (version 12.0.9). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates.

Determination of Metabolites Produced by the Most Potent Endophytic Fungal Strain:

A. tenuissima SN2 exhibited the highest reduction activity against AFB1 and CIT, so it was utilized for further studies. Five mL of the freshly prepared inoculum was inoculated into 2000 mL Erlenmeyer flask containing 1000 mL of CYA broth medium then incubated in shaking incubator at 150 rpm for 10 days in the dark at 28°C. After incubation, the filtrate was extracted with equal volume of ethyl acetate (EtOAc) three successive times. The EtOAc phase was filtered over anhydrous sodium sulfate, then evaporated under vacuum using rotary evaporator (Bhardwaj *et al.,* 2015). According to Yan *et al.* (2004) with minor modifications, the crude extract was resuspended in absolute methanol and spotted on TLC plates, which were then developed in chloroform: methanol (93:7, v/v) solvent system. The bands observed on the TLC plates under UV light were scrapped off and eluted in methanol for centrifugation. The methanol was evaporated till dryness, and the obtained dried extracts were used for purification by column chromatography.

Purification of the Bands Extracted from *A. tenuissima* SN2 Using Column Chromatography:

The dried extracts were resuspended in 5 mL of absolute methanol and then subjected to solid-phase extraction (SPE) column packed with silica gel bonded to C18 (Zheng *et al.,* 2006). The sample extract was forced through an SPE column using a rubber syringe plunger, allowing impurities to be retained while the purified extract was collected in a vial (Malone *et al.,* 1998).

Effect of Metabolites Extracted from *A. tenuissima* SN2 on AFB1 and CIT Production:

The purified extract of each band was resuspended in dimethyl sulfoxide (DMSO) to achieve a concentration of 100 µg/mL (Lv *et al.*, 2010)**.** One mL of freshly prepared inoculum of AFB1 and CIT producing fungi, were separately inoculated into 250 mL Erlenmeyer flasks containing 50 mL of CYA broth medium amended with 100 µg/mL of each purified band and incubated for 10 days in the dark at 28°C. Flasks singly inoculated with AFB1 and CIT producing fungi were used as controls. After incubation, the cultures were filtered through pre-weighed Whatman No. 1 filter papers. For determination the concentration of AFB1 and CIT, the filtrates were extracted, quantified spectrophotometrically and the percentage of reduction of mycotoxins was calculated (as mentioned earlier). All assays were performed in triplicate.

Gas Chromatography –Mass Spectrometry (GC-MS) Analysis of The Most Potent Band Extracted from *A. tenuissima* SN2:

The purified band (2) with the highest mycotoxin reduction was subjected to GC/MS analysis. Samples were derivatized following a modified Fiehn *et al.* (2000) method, which involved resuspending dried samples in methoxyamine hydrochloride for oximation (90 min) followed by silylation with bis (trimethylsilyl) trifluoroacetamide (BSTFA): trimethylchloro-silane (TMCS) (99:1). The GC/MS system (Agilent Technologies 7890B/5977A, Central Laboratories Network, National Research Centre, Cairo, Egypt) was equipped with a DB-5MS column (30 m x 0.25 mm ID, 0.25 µm film). Analyses used hydrogen carrier gas (1.0 mL/min, splitless 1 µL injection). The oven temperature program initiated at 60°C (1 min), ramped at 10°C/min to 320°C (10 min hold), with injector and detector at 300°C and 320°C, respectively. Mass spectra (EI, 70 eV, m/z 50-600, 6 min solvent delay) were obtained, and metabolites were identified by comparing mass spectra with Wiley and NIST Library databases.

AFB1 Biosorption Assay:

Preparation of the Endophytic *M. guilliermondii* UD2 Cells:

The yeast strain used in this experiment was previously isolated from the leaves of *U. dioica* plant. Yeast inoculum was prepared according to Chlebicz and Śliżewska (2020) with minor modifications. Yeast was cultivated on yeast extract peptone dextrose (YPD) agar media then incubated for 48 h at 30°C. After incubation, the yeast strain was aseptically transferred to YPD broth media and incubated in shaker at 120 rpm for 24 h at 30°C, after which, cells were collected by centrifugation at 3468×g for 10 min. Subsequently, the supernatant was discarded, and the yeast biomass was washed three times with sterilized distilled water to remove any residual media and re-centrifuged. The obtained pellets were then diluted with sterilized distilled water to achieve a cell concentration of 1.0×10^7 cells/mL using hemocytometer. To prepare heat-inactivated yeast, the cells were boiled for 1 h at 100°C.

Preparation of AFB1 Working Solution:

The AFB1, which was previously separated from TLC plates, and the standard solid AFB1 (Sigma Aldrich, Taufkirchen, Germany) were separately dissolved in acetonitrile to obtain a stock solution of AFB1 at 10 mg/mL. The acetonitrile was evaporated under vacuum, and AFB1 was resuspended in methanol. The working solution of 5 µg/mL AFB1 was prepared in PBS at pH 7.0 utilizing a stock solution of AFB1 in methanol (El-Nezami *et al.,* 1998; Haskard *et al.,* 2001; Topcu *et al.,* 2010).

Biosorption Experiment:

Biosorption of AFB1 from Phosphate-buffered saline (PBS) was performed as explained by Topcu *et al.,* (2010) and Bovo *et al.* (2013) with minor modifications. The assay was tested under the influence of different factors such as yeast viability, pH and incubation period. Yeast pellets either viable or heat-inactivated (1.0×10^7) were suspended in 3 ml of PBS spiked with 5 µg/mL of AFB1 and incubated for 6, 12, and 24 h at 30°C and pH 7.0 in shaking incubator. The effect of pH on the binding of AFB1 in PBS medium was tested at 30°C after 24 h. The pH of PBS containing AFB1 was adjusted to 3.0, 5.0, and 7.0 with 1M HCl using pH meter. AFB1 solution without yeast cells served as control. After incubation, all samples were centrifuged at 1800×g for 15 min and the supernatants containing AFB1 residues were quantified spectro-photometrically (as mentioned earlier). The biosorption efficiency of AFB1 was calculated using the following equation:

$$\% \text{ Reduction} = (C_0 - C / C_0) \times 100$$

where C_0 and C refer to the initial and residual concentrations of AFB1, respectively.

Statistical Analysis:

Data were analyzed using one-way analysis of variance (ANOVA) with Duncan's test at $P \leq 0.05$, conducted through the Statistical Package for Social

Sciences (SPSS) software, version 27. Results are represented as the mean ± standard deviation of triplicate samples.

RESULTS

Isolation and Morphological Identification of Isolated Fungal Species: From Canned Food Products:

A total of twenty-two fungal species were isolated from twelve samples of seven different canned food products. They all belonged to the *Aspergillus* genus. Among the tested canned foods, tuna exhibited the highest fungal contamination. It was followed by smoked tuna, sardine, and mackerel. Anchovy showed less contamination, and bully beef was the least contaminated. Notably, Salmon can was entirely free from any fungal contamination (Fig.1). The distribution of fungal isolates showed *A. niger* to be the most prevalent species. *A. fumigatus* was the second most frequent, followed by *A. terreus*. The least common isolates observed were *A. carbonarius* and *A. flavus* (Fig. 2).

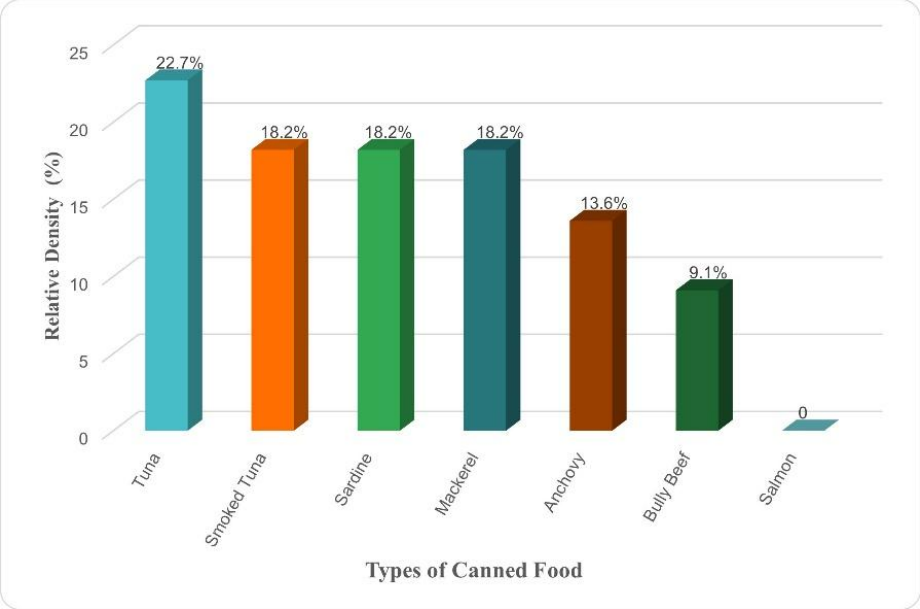

Fig. 1 Relative density of fungal contamination in surveyed canned food products.

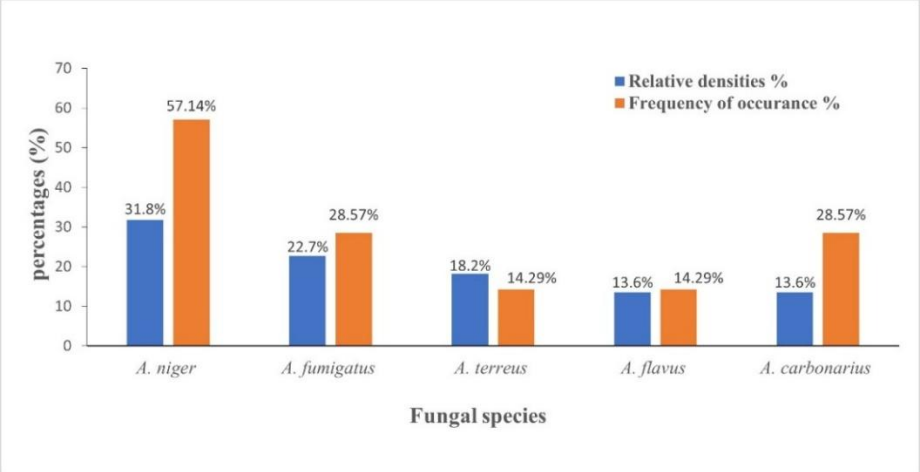

Fig. 2 Relative density and frequency of occurrence of fungi isolated from canned food products.

From Medicinal Plants:

A total of thirty-six endophytic fungal isolates were isolated from two medicinal plants, *S. nigrum* and *U. dioica* plant. According to morphological identification, they belonged to five genera

Alternaria, Fusarium, Meyerozyma, Stemphylium, and *Curvularia*. Table 1, showed the frequency percentages of each fungal isolate.

Table 1. Diversity and abundance of endophytic fungi isolated from *Solanum nigrum* and *Urtica dioica* leaves and stems.

Fungal isolates	*S. nigrum*		*U. dioica*		Total isolates	Relative density %	Frequency of occurrence %
	Leaf	Stem	Leaf	Stem			
Alternaria chlamydospora SN1	3	-	-	-	3	18.75	25
A. tenuissima SN2	2	-	-	-	2	12.5	25
Fusarium culmorum SN3	-	1	-	-	1	6.25	25
F. solani SN4	-	1	-	-	1	6.25	25
A. alternata UD1	1	-	1	-	2	12.5	50
Meyerozyma guilliermondii UD2	-	-	-	2	2	12.5	25
Curvularia aeria UD3	-	-	-	1	1	6.25	25
F. dimerum UD4	1	-	-	2	3	18.75	50
Stemphylium vesicarium UD5	-	-	1	-	1	6.25	25
Total count	7	2	2	5	16	100	

Detection of AFB1 and CIT using TLC:

Fungal species isolated from various canned food products were examined for their potentiality to produce AFB1 and CIT using TLC analysis (Table 2). It has been observed that only *A. flavus* ST1 produced AFB1 at Rf (0.58), and *A. terreus* S1 produced CIT at Rf (0.41).

Table 2. Detection of AFB1 and CIT produced by canned food fungi.

Canned food samples	Fungal isolates	AFB1 and CIT positive isolates
Tuna	*A. niger* T1	N/D
	A. fumigatus T2	N/D
Smoked tuna	*A. flavus* ST1	AFB1
	A. carbonarius ST2	N/D
Anchovy	*A. niger* A1	N/D
	A. carbonarius A2	N/D
Sardine	*A. terreus* S1	CIT
Mackerel	*A. niger* M1	N/D
	A. fumigatus M2	N/D
Bully beef	*A. niger* BB1	N/D

N/D = not detected

Effect of Endophytic Fungi on AFB1 and CIT Production:

The effect of endophytic fungal species on the AFB1 and CIT production is presented in Figure 3. The study showed that the endophytic fungi greatly reduce the production of CIT than AFB1. *A. tenuissima* SN2 was the most effective endophytic fungus in reducing both mycotoxins, achieving a reduction of 74.51% for CIT and 24.64% for AFB1, while *S. vesicarium* UD5 had the lowest reducing activity, with only 4.56% of AFB1 and 3.46% of CIT. The other endophytic fungi exhibited different reducing rates against both mycotoxins. Consequently, the

endophytic *A. tenuissima* SN2 was selected for metabolite identification to determine the compounds responsible for the observed reduction in mycotoxin concentrations. Similarly, the endophytic *M. guilliermondii* UD2, which was the second most effective isolate in reducing AFB1, was chosen for the AFB1 binding experiment.

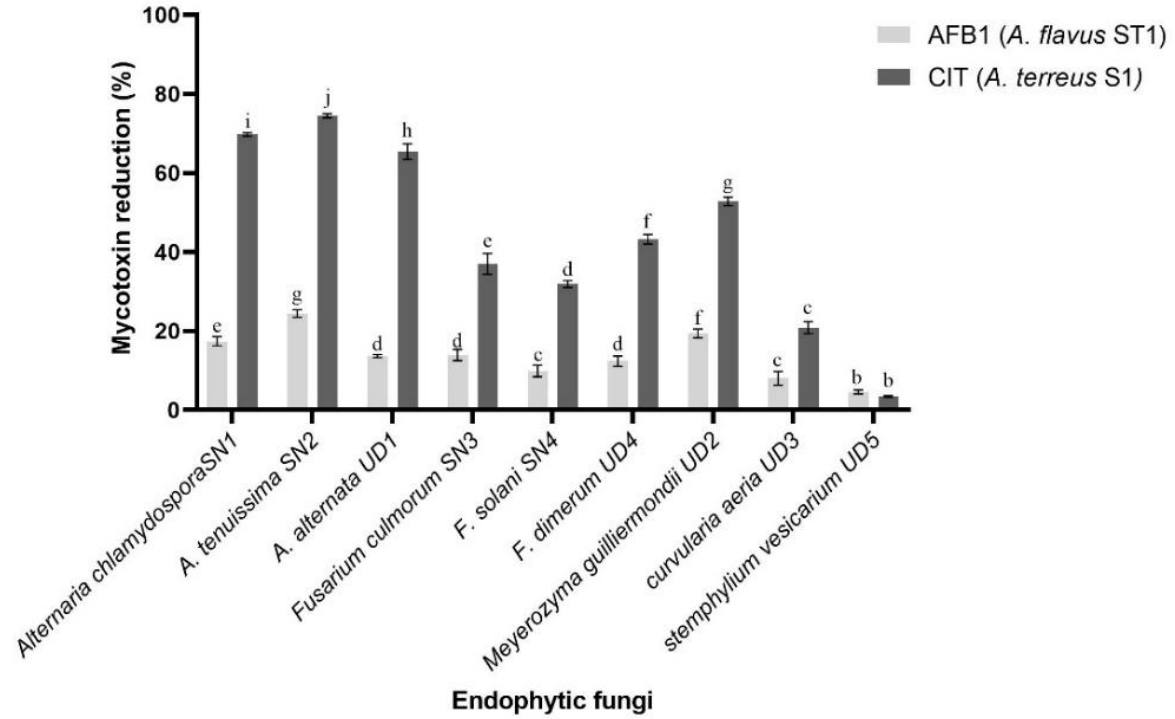

Fig. 3 Reduction of AFB1 (produced by *A. flavus* ST1) and CIT (produced by *A. terreus* S1) by endophytic fungal species.

Molecular Identification of The Selected Fungal Isolates:

The most potent endophytic fungal species, *A. tenuissima* SN2 and *M. guilliermondii* UD2, along with the fungi producing CIT and AFB1, *A. terreus* S1 and *A. flavus* ST1, respectively, were molecularly identified and their sequences were compared with those available in the public GenBank databases of the NCBI using the BLAST alignment search program. Accession numbers of the identified fungi were obtained as PQ517551 for *A. tenuissima,* PV660158 for *M. guilliermondii*, PQ518559 for *A. terreus,* and PQ517527 for *A. flavus.* The phylogenetic trees of the identified fungal species were displayed in (Fig. 4a-d, respectively).

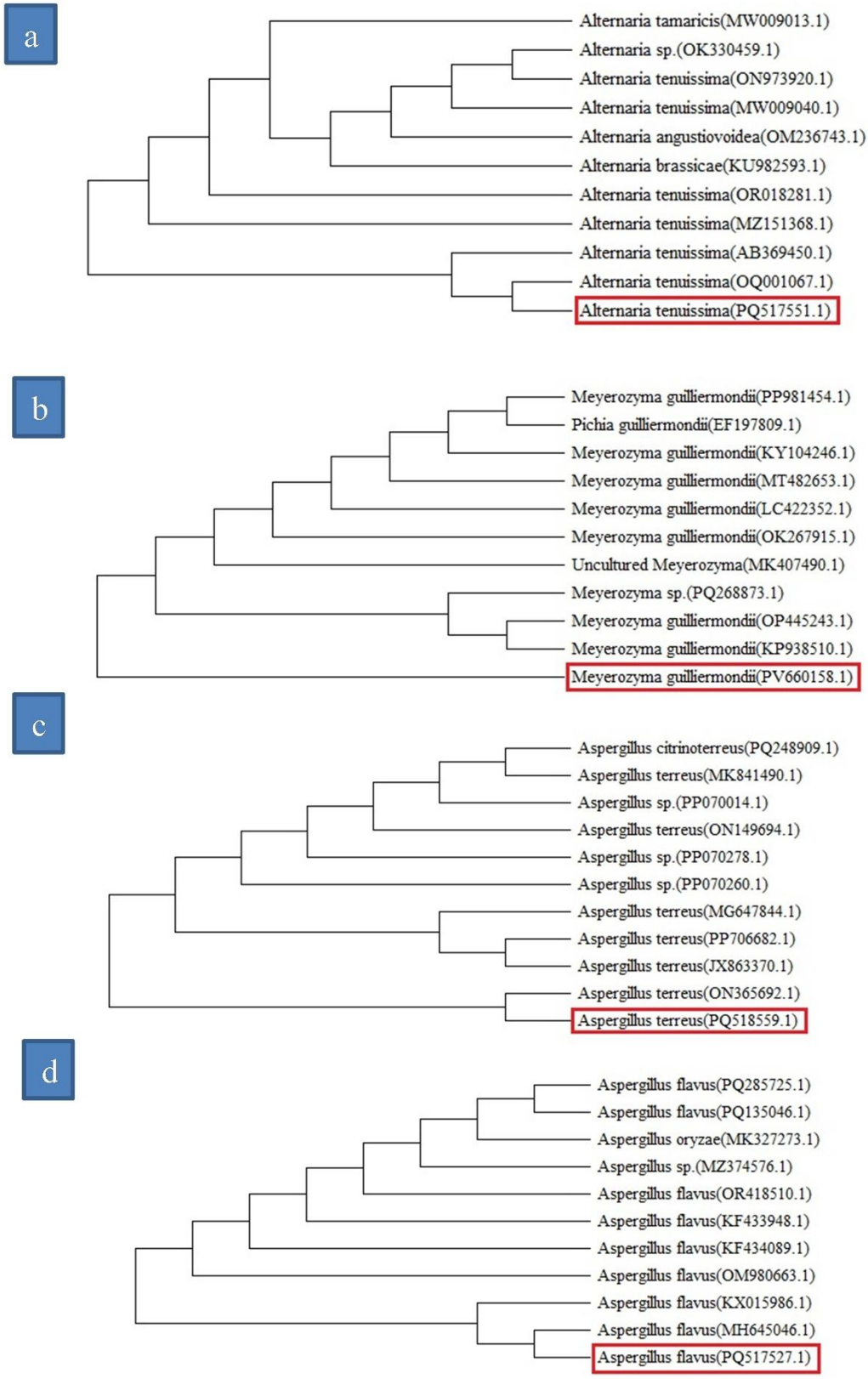

Fig. 4 The phylogenetic analyses of *A. tenuissima* PQ517551.1 (a), *M. guilliermondii* PV660158.1 (b), *A. terreus* PQ518559.1 (c), and *A. flavus* PQ517527.1 (d).

Effect of Metabolites Extracted from *A. tenuissima* SN2 on AFB1 and CIT Production:

The EtOAc extract of *A. tenuissima* SN2 was separated into four distinct bands via TLC. These bands were subsequently purified to assess their individual impact on AFB1 and CIT production. As shown in Table 3, all four purified bands significantly reduced CIT production by 86.47%, 90.17%, 78.12%, and 73.91%, respectively. In contrast, while bands (2) and (3) exhibited AFB1 detoxification with removal percentages of 21.09% and 11.22%, respectively, bands (1) and (4) increased AFB1 production by 23.89% and 21.21%, respectively.

Table 3. Effect of the four bands produced by *A. tenuissima* SN2 on AFB1 and CIT production.

Extracted bands	Removal %		Stimulation %
	AFB1	CIT	AFB1
1	-	86.47 ± 0.60^{c}	23.89 ± 0.62^{d}
2	21.09 ± 1.05^{b}	90.17 ± 0.38^{d}	-
3	11.22 ± 0.99^{a}	78.12 ± 0.79^{b}	21.21 ± 0.80^{c}
4	-	73.91 ± 0.65^{a}	-

The values represent the mean of triplicates ± SD; dissimilar letters within columns are significantly different ($p \leq 0.05$).

GC-MS Analysis of The Most Potent Band (2) Extracted from *A. tenuissima* SN2:

Among the four purified bands, band (2) at Rf (0.26) gave the highest detoxifying activity, reducing CIT by 90.17% and AFB1 by 21.09%. Therefore, it was analyzed by GC/MS. The analysis identified 12 bioactive metabolites detected at different retention times (RT) with varying area percentages (Table 4).

Table 4. The presence of bioactive metabolites in the band (2) extracted from *A. tenuissima* SN2 detected through GC-MS.

Metabolite name	Molecular formula	Retention time (min)	Area%
Heptanoic acid	$C_7H_{14}O_2$	7.725	1.38
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	12.31	6.14
Suberic acid	$C_8H_{14}O_4$	14.193	6.35
Azelaic acid	$C_9H_{16}O_4$	15.125	12.65
Sebacic acid	$C_{10}H_{18}O_4$	16.264	0.65
Decanoic acid	$C_{11}H_{22}O_2$	17.277	2.36
Palmitic acid	$C_{16}H_{32}O_2$	17.746	3.33
α-Linolenic acid	$C_{18}H_{30}O_2$	18.109	3.4
Oleic Acid	$C_{18}H_{34}O_2$	19.297	1.51
Stearic acid	$C_{18}H_{36}O_2$	19.529	1.82
Oleamide	$C_{18}H_{35}NO$	22.876	1.19
Monopalmitin	$C_{19}H_{38}O_4$	23.682	9.97

Biosorption of AFB1 from Aqueous Solution by *M. guilliermondii* UD2:

The impact of incubation period and pH on AFB1 binding by viable and heat-treated cells are given in Table 5. Both cells exhibited high binding abilities for AFB1 under all tested conditions. Viable cells reduced the concentration of AFB1 by 87.42%, 85.68% and 79.47% after 6, 12, and 24 h, respectively, while heat-treated cells reduced the concentration of AFB1 by 84.68%, 81.61% and 77.19% after the same time periods. It was observed that as the time of incubation increased, the removal

percentages of AFB1 decreased. Results also indicated that the maximum removal of AFB1 was achieved at pH 7.0, at which, viable cells reduced 80.07% while heat-treated cells reduced 77.79% of AFB1. Both cells exhibited high removal percentages at pH 3.0 compared to pH 5.0. However, viable cells had a better binding capability at different incubation periods and pH values than heat-treated cells.

Table 5. Effect of incubation period and pH on the removal of AFB1 (5 µg/ml) by *M. guilliermondii* UD2 cells in PBS solution at 30°C.

Removal % of AFB1 by *M. guilliermondii* UD2 cells		
	Viable	**Heat-treated**
Incubation period (h)		
6	87.42 ± 0.11^{c}	84.68 ± 0.31^{c}
12	85.68 ± 0.29^{b}	81.61 ± 0.24^{b}
24	79.47 ± 0.22^{a}	77.19 ± 0.14^{a}
pH		
3.0	69.03 ± 0.32^{b}	66.82 ± 0.33^{b}
5.0	54.32 ± 0.36^{a}	48.56 ± 0.29^{a}
7.0	80.07 ± 0.28^{c}	77.79 ± 0.25

The values represent the mean of triplicates ± SD; dissimilar letters within columns are significantly different from each other ($p \leq 0.05$).

DISCUSSION

Mycotoxins, commonly found throughout the food chain, cause various harmful effects in both animals and humans. (Li *et al.,* 2025). Scientists have been investigating various strategies to control mycotoxins in food and feed. Although the discovery of several treatments that can reduce the concentrations of certain mycotoxins, no single technique has proven to be equally effective against the diverse range of mycotoxins that can contaminate various commodities (Abrunhosa *et al.,* 2010). Foods that are commercially canned are considered to be safe because they are processed under strict standards; however, if processing techniques are not followed correctly, microbial infections may result (Nasser, 2015). Such contamination may also arise from improper handling, storage, distribution, marketing, and transportation throughout the food supply chain (Effiuvwevwere, 2000). Since the sources of these contaminants are varied, it is difficult to determine the specific stage at which contamination occurred (Bockelmann *et al.,* 2008). Taiwo's (2020) investigation into microbial contamination in canned dairy foods revealed the presence of fungal isolates in both expired and unexpired samples, indicating that fungal growth can occur even before the expiration date.

In the present study, five fungal species were isolated from unexpired canned foods including, *A. niger, A. fumigatus, A. flavus, A. terreus,* and *A. carbonarius*. *A. flavus* ST1 *and A. terreus* S1 were observed to produce AFB1 and CIT. This study investigated the potential of endophytic fungi to prevent or reduce mycotoxin production by fungi found in canned foods. Endophytic fungi offer a promising and relatively unexploited source of secondary metabolites, relevant not only to mycotoxin control but also to various industrial applications (Sharma *et al.,* 2016; Ismaiel and Ali, 2017; Ismaiel *et al.,* 2023). In the present study, nine endophytic fungi were isolated from *Solanum nigrum* and *Urtica dioica* plants. Diverse rates of reduction in AFB1 and CIT production were observed across all tested endophytic fungal isolates. The observed reduction in mycotoxin production is likely due to competitive interactions between the endophytic and mycotoxigenic fungal species. This interaction may be mediated by anti-mycotoxin compounds produced by the endophytic fungi, which can either

inhibit the growth of mycotoxigenic fungi or disrupt their mycotoxin biosynthesis pathways, thereby decreasing mycotoxin levels in the growth media.

The results indicated that *A. tenuissima* PQ517551 was the most effective endophytic fungus in reducing CIT, while exhibiting minimal impact on AFB1. GC/MS analysis of the most potent extract (band 2) from *A. tenuissima* revealed the presence of 12 bioactive metabolites, including five fatty acids: decanoic acid, palmitic acid, α-linolenic acid, stearic acid, and oleic acid. These fatty acids have recently been reported as potential anti-mycotoxins (Tiwari *et al.,* 1986; Hajjaj *et al.,* 2000; Altieri *et al.,* 2007; Panda and Ali, 2012; Qiu *et al.,* 2021). Qiu *et al.* (2021) reported that fatty acids and their derivatives have been identified as promising natural antifungal and anti-mycotoxin agents. They may provide novel strategies for controlling fungal growth and mycotoxin production in food. Although the fungistatic and fungicidal properties of many short and long-chain fatty acids have been examined, their effectiveness in controlling spoilage molds in food and their specific mechanism of action remain unknown and not well understood (Walters *et al.* 2003, 2004). The presence of lauric, palmitic, and oleic acids in synthetic media decreased aflatoxin production (10–75.8%) and mycelium mass, whereas linolenic and linoleic acids significantly increased aflatoxin production (1070–3430%, respectively) (Tiwari *et al.*, 1986). In contrast, Priyadarshini and Tulpule (1980) found that myristic, palmitic, and stearic acids stimulated the production of AFB1, while oleic acid and linoleic acid, seemed to inhibit AFB1 synthesis. Similarly, Yan *et al.* (2014) and Hamid and Smith (1987) reported that palmitic acid and stearic acid stimulate AF production. Yan *et al.* (2014) also found that linolenic acid cause inhibition in AF production after several hours of exposure to air, suggesting that this inhibition resulted from the suppression of gene expression within the AF biosynthetic gene cluster, accompanied by an increase in sporulation, mycelial growth, and kojic acid production. In the present study, the GC/MS analysis revealed the presence of oleic, palmitic, stearic and α-linolenic acids in band (2), this may explain why the reduction of AFB1 did not exceed 21.09% as the extract contained both inhibitory and stimulatory fatty acids. Whereas the concentration of CIT was significantly reduced as GC/MS also revealed the presence of decanoic acid in band (2). The results in this study were consistent with Hajjaj *et al.* (2000) studies, who observed that medium chain fatty acids, including octanoic, decanoic, and dodecanoic acids inhibit CIT production more than fatty acids with longer or shorter carbon chain. Additionally, Panda and Ali (2012) reported that decanoic acid was highly effective in reducing CIT in the solid fermentation medium of red mold rice. According to the recent studies, the impact of fatty acids on mycotoxins depends on the type and concentration of fatty acid used.

Various biological studies have been developed to eliminate different mycotoxins from liquid media or commercial beverages using adsorption techniques. These methods use either organic (shrimp shells, chitin) or biological (bacteria, biofilm, and yeast) binders (Taheur *et al.,* 2017; Chlebicz and Śliżewska, 2020). Yeasts are capable of binding mycotoxins because of their cell wall components, particularly the glucomannans (Dawson *et al.,* 1999; Poloni *et al.,* 2015). *Meyerozyma guilliermondii*, a yeast recognized for its potential in AFB1 reduction, has been previously isolated as an endophyte from diverse plant sources. For example, Ling *et al.* (2019), identified its presence in tangerine peels, while Peng *et al.* (2018) reported its high prevalence within citrus pulp. In the present study, *M. guilliermondii* PV660158.1 isolated from *Urtica dioica* was investigated for its AFB1-binding capacity in PBS, revealing significant AFB1 reduction rates, ranging

from 48.56% to 87.42%. *M. guilliermondii* AF01 was characterized as a strain capable of AFB1 removal through both adsorption and degradation mechanisms (Zhang *et al.*, 2023). These results are consistent with those of Zhang *et al.* (2024), who demonstarted that *M. guilliermondii* AF01 strongly removed AFB1 from peanut meal (over 70% within 3 days of fermentation) without affecting its quality. In addition, the mechanism of AFB1 degradation by *M. guilliermondii* AF01 was elucidated by Zhang *et al.* (2025). Dikmetas *et al.* (2023), also reported that *M. guilliermondii* could inhibit the mycelial growth of *A. flavus* and decrease AFB1 production. Additionally, the effective degradation of patulin by *M. guilliermondii* suggests a novel approach for the detoxification of this mycotoxin as well (Fu *et al.*, 2021).

The viability of the yeast did not significantly influence the reduction of AFB1, however, viable cells exhibited a slightly higher reduction rates than heat-treated cells across all tested conditions. Istiqomah, *et al.* (2019) reported that viable cells of *S. cerevisiae* B18 exhibited higher AF binding percentage (71.86%) than non-viable cells (69.52%). However, they differ from the findings reported by Shetty *et al.* (2007) and Lee *et al.* (2003) who found that heat treatment of cells enhanced their binding capabilities among 20-50%. However, Pizzolitto *et al.* (2011) found that both viable and heat-treated cells of various strains of yeasts and lactic acid bacteria can bind AFB1 with similar efficiency, suggesting that the mechanism involved did not require metabolic conversion of the toxin by cells. Additionally, Topcu *et al.*, (2010) determined that the effectiveness of mycotoxin removal from aqueous solutions varies across different strains, independent of cell viability.

In the present study, results indicated that both viable and heat-treated cells effectively bound AFB1 from PBS after 6 h of incubation, with reduction rates reaching 87.42% and 84.68%, respectively. It was observed that as the incubation period increased, the reduction of AFB1 decreased, reaching 79.47% and 77.19% after 24 h, respectively. In agreement with our results, Topcu *et al.*, (2010) revealed that after 24 h, some of AFB1 may be released back into the solution from the bacterium-AFB1 complex, since AFB1 was not tightly bound to the viable bacterial cells. In similar, Haskard *et al.*, (2001), stated that the stability of the complexes is dependent on microbial strains, treatment, and environmental conditions. Furthermore, Bueno *et al.* (2007) explained that the binding process of AFB1 to microorganisms was fast (within 1 min) and involved the development of a reversible complex between the toxin and microbial surface, with no chemical alteration of the toxin.

The impact of pH on binding AFB1 by both viable and heat-treated *M. guilliermondii* cells showed that the highest reduction of AFB1 was achieved at pH 7.0 (80.07% and 77.79%, respectively), while the least was at pH 5.0 (54.32% and 48.56%, respectively). Accordingly, Ul Hassan *et al.*, (2023) found that different *S. cerevisiae* strains exhibited the highest AFB1 binding activities at pH 7.0. In addition, Hathout (2023) reported that the mean percentages of AFB1 bound to two strains of *S. cerevisiae* ranged from 21% to 99.40% at pH 7.0. In contrast, Kong *et al.* (2014) found that in an in vitro experiment which mimicked the pH condition of a pig's gastrointestinal tract (pH 2.0), yeast cell wall product had adsorbed 92.7% of AFB1 after 2 h of incubation. The variations in the adsorption of AFB1 may be related to the nature of yeast, incubation time, and/or medium pH (Ul Hassan *et al.*, 2023).

Conclusion

It was concluded that the endophytic fungi were effective in reducing CIT and AFB1 produced by mycotoxigenic fungi isolated from canned food products. *A. tenuissima* PQ517551.1 had a significant ability to reduce CIT and exhibited minimal effect on AFB1 in liquid media. GC/MS analysis of its purified extract (band 2)

revealed the presence of five fatty acids, which might be responsible for the reduction of CIT and AFB1 production. Furthermore, the endophytic *M. guilliermondii* PV660158 (viable and heat-treated) cells were efficiently reducing AFB1 in PBS under all tested conditions. While this study demonstrates the promising potential of endophytic fungi for biological decontamination of mycotoxins, future investigations are crucial for elucidating the control mechanisms of endophytic fungi against toxigenic fungi and for confirming their safety through extensive toxicological analysis.

Declarations:

Ethical Approval: Not applicable.

Authors Contributions: T.M.A.A. and M.A.S. conceptualized the study. R.M.A.A., T.M.A.A., and A.A.I. carried out the methodology, data collection, and analysis. R.M.A.A. prepared the initial manuscript draft. T.M.A.A., A.A.I., and M.A.S. reviewed and edited the manuscript. All authors approved the final published version.

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REFERENCES

Abd-Allah E. F., & Ezzat, S. M. (2005). Natural occurrence of citrinin in rice grains and its biocontrol by *Trichoderma hamatum*. *Phytoparasitica*, *33*, 73–84. https://doi.org/10.1007/BF02980928

Abrunhosa, L., Paterson, R. R. M., & Venâncio, A. (2010). Biodegradation of ochratoxin A for food and feed decontamination. *Toxins*, *2*(5), 1078–1099. https://doi.org/10.3390/toxins2051078

Ahima, J., Zhang, H., Apaliya, M. T., Zhang, X., Yang, Q., & Zhao, L. (2019). The effect of *Rhodotorula mucilaginosa* on degradation of citrinin production by *Penicillium digitatum* and its toxin in vitro. *Journal of Food Measurement and Characterization*, *13*(4), 2998–3004. Springer US. Retrieved from https://doi.org/10.1007/s11694-019-00220-6

Ali, N., Blaszkewicz, M., Mohanto, N. C., & Rahman, M. (2015). First results on citrinin biomarkers in urines from rural and urban cohorts in Bangladesh. *Mycotoxin Res* 31, 9–16. https://doi.org/10.1007/s12550-014-0217-z

ALKahtani, M. D. F., Fouda, A., Attia, K. A., Al-Otaibi, F., Eid, A. M., El-Din Ewais, E., Hijri, M., St-Arnaud, M., El-Din Hassan, S., Khan, N., Hafez, Y. M., & Abdelaal, K. A. A. (2020). Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. *Agronomy*, *10*(9), 1–18. https:// doi. org/ 10. 3390/ agronomy10091325

Altieri, C., Cardillo, D., Bevilacqua, A., & Sinigaglia, M. (2007). Inhibition of *Aspergillus* spp. and *Penicillium* spp. by fatty acids and their monoglycerides. *Journal of Food Protection*, *70*(5).1201212. https://doi. org/ 10.4315/ 0362-028X-70.5.1206

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990).

Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3).403-410. https://doi. org/ 10.1016/S0022-2836(05)80360-2

Assaf, J. C., Khoury, A. El, Chokr, A., Louka, N., & Atoui, A. (2019). A novel method for elimination of aflatoxin M1 in milk using *Lactobacillus rhamnosus* GG biofilm. *International Journal of Dairy Technology*, *72*(2), 248–256.

Baiyewu, R. A., Amusa, N. A., Ayoola, O. A., & Babalola, O. O. (2007). Survey of the post harvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya L*) in South Western Nigeria. *African Journal of Agricultural Research*, *2*(4), 178–181. http://www.academicjournals. org/AJAR

Bhardwaj, A., Sharma, D., Jadon, N., & Agrawal, P. K. (2015). Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus roxburghii*. *Archives of Clinical Microbiology 6*(3), 1–9.

Bockelmann, W., Heller, M., & Heller, K. J. (2008). Identification of yeasts of dairy origin by amplified ribosomal DNA restriction analysis (ARDRA). *International Dairy Journal*, *18*(10–11). https:// doi.org/10.1016/j.idairyj.2008.05. 008

Bovo, F., Corassin, C. H., Rosim, R. E., & de Oliveira, C. A. F. (2013). Efficiency of Lactic Acid Bacteria Strains for Decontamination of Aflatoxin M1 in Phosphate Buffer Saline Solution and in Skimmed Milk. *Food and Bioprocess Technology*, *6*(8), 2230–2234. https://doi.org/10.1007/s11947-011-0770-9

Bueno, D. J., Casale, C. H., Pizzolitto, R. P., Salvano, M. A., & Oliver, G. (2007). Physical adsorption of aflatoxin B1 by lactic acid bacteria and *Saccharomyces cerevisiae*: A theoretical model. *Journal of Food Protection*, *70*(9):2148-2154. https://doi. org/ 10.4315/ 0362-028X-70.9.2148

Bzducha-Wróbel, A., Bryła, M., Gientka, I., Błażejak, S., & Janowicz, M. (2019). Candida utilis atcc 9950 cell walls and β(1,3)/(1,6)-glucan preparations produced using agro-waste as a mycotoxins trap. *Toxins*, *11*(4).192. https://doi.org/10. 3390/toxins11040192

Chlebicz, A., & Śliżewska, K. (2020). In Vitro Detoxification of Aflatoxin B1, Deoxynivalenol, Fumonisins, T-2 Toxin and Zearalenone by Probiotic Bacteria from Genus *Lactobacillus* and *Saccharomyces cerevisiae* Yeast. *Probiotics and Antimicrobial Proteins*, *12*(1), 289–301. https://doi.org/10. 1007/ s12602-018-9512-x

Dai, C., Tian, E., Hao, Z., Tang, S., Wang, Z., Sharma, G., Jiang, H., & Shen, J. (2022). Aflatoxin B1 Toxicity and Protective Effects of Curcumin: Molecular Mechanisms and Clinical Implications. *Antioxidants*, *11* (10).2031. https://doi.org/10. 3390 / antiox11102031.

Dawson, K. A., Evans, J., & Kudupoje, M. (1999). Understanding the adsorption characteristics of yeast cell wall preparations associated with mycotoxin binding. *North*, 169–182.

Dikmetas, D. N., Özer, H., & Karbancıoglu-Guler, F. (2023). Biocontrol Potential of Antagonistic Yeasts on In Vitro and In Vivo Aspergillus Growth and Its AFB1 Production. *Toxins*, *15*(6):402. https://doi.org/10. 3390/ toxins15060402

Effiuvwevwere, B. J. O. (2000). Microbial spoilage agents of Tropical and assorted fruits and Vegetables (An Illustrated References Book). *Port*

Harcourt: Paragraphics Publishing Company, 1–39.

El-Nezami, H., Kankaanpaa, P., Salminen, S., & Ahokas, J. (1998). Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. *Food and Chemical Toxicology*, *36*(4):321-326. https: //doi.org/10.1016/S0278-6915 (97) 00160-9

El-Shanawany, A. A., Eman Mostafa, M., & Barakat, A. (2005). Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic *Aspergilli* toxins. *Mycopathologia*, *159*(2), 281–289. https://doi. org/ 10. 1007/s11046-004-5494-1

Farawahida, A. H., Palmer, J., & Flint, S. (2022). International Journal of Food Microbiology *Monascus* spp. and citrinin: Identification, selection of *Monascus* spp. isolates, occurrence, detection and reduction of citrinin during the fermentation of red fermented rice. *International Journal of Food Microbiology*, *379*(June), 109829. https://doi.org/10.1016/j.ijfoodmi cro.2022.109829

Fiehn, O., Kopka, J., Dörmann, P., Altmann, T., Trethewey, R. N., & Willmitzer, L. (2000). Metabolite profiling for plant functional genomics. *Nature Biotechnology*, *18*(11):1157-1161. https://doi. org/10.1038/81137

Fovo, F. P., Maeda, D. G., & Kaale, L. D. (2025). Microbiological approaches for mycotoxin decontamination in foods and feeds to enhance food security: a review. *Mycotoxin Research,* 41 (2):385-404. doi: 10.1007/s12550-025-00587-0. Epub 2025 Mar 24. PMID: 40126858.

Fu, Y., Yang, Q., Solairaj, D., Godana, E. A., Routledge, M. N., & Zhang, H. (2021). Biodegradation of mycotoxin patulin by the yeast *Meyerozyma guilliermondii*. *Biological Control*, *Vol.160*. https://doi.org/10.1016/j.biocontr ol.2021.104692

Gilman, J. C. (1957). A Manual of Soil Fungi-2nd Edifion. Iowa State Coll. Press, Ames.

Hajjaj, H., Klaébé, A., Goma, G., Blanc, P. J., Barbier, E., & François, J. (2000). Medium-chain fatty acids affect citrinin production in the filamentous fungus *Monascus ruber*. *Applied and Environmental Microbiology*, *66*(3). https://doi. org/10. 1128/ AEM. 66.3. 1120-1125. 2000

Hamid, A. Bin, & Smith, J. E. (1987). Effect of exogenous lipids on growth and aflatoxin production by *Aspergillus flavus*. *Transactions of the British Mycological Society*, *89*(3).384-387. https://doi.org/10.1016/ s0007-1536(87)80123-7

Haskard, C. A., El-Nezami, H. S., Kankaanpää, P. E., Salminen, S., & Ahokas, J. T. (2001). Surface Binding of Aflatoxin B1 by Lactic Acid Bacteria. Applied and Environmental Microbiology, 67(7). https://doi.org/10.1128/ AEM.67.7.3086-3091.2001

Hathout, A., & Abdel-Nasser, A. (2023). The efficiency of *Saccharomyces cerevisiae* as an antifungal and antimycotoxigenic agent. *Biointerface Research in Applied Chemistry*,*13*(4), 354.

He, Y., & Cox, R. J. (2016). The molecular steps of citrinin biosynthesis in fungi. *Chemical science*, *7*(3), 2119–2127. Royal Society of Chemistry.

Hertwig, A. M., Iamanaka, B. T., Amorim Neto, D. P., Rezende, J. B. de, Martins, L. M., Taniwaki, M. H., & Nascimento, M. S. (2020). Interaction of *Aspergillus flavus* and *A. parasiticus* with

Salmonella spp. isolated from peanuts. *International Journal of Food Microbiology*, *328* (December 2019), 108666. https: //doi.org/10.1016/j.ijfoodmicro.20 20.108666

Huang, Z., Zhang, L., Gao, H., Wang, Y., Li, X., Huang, X., & Huang, T. (2019). Soybean isoflavones reduce citrinin production by *Monascus aurantiacus* Li AS3.4384 in liquid state fermentation using different media. *Journal of the Science of Food and Agriculture*, *99*(10), 4772–4780. https://doi.org/10.1002/jsfa.9723

Ismaiel, A A, & Tharwat, N. A. (2014). Antifungal activity of silver ion on ultrastructure and production of aflatoxin B1 and patulin by two mycotoxigenic strains, *Aspergillus flavus* OC1 and *Penicillium vulpinum* CM1. *Journal de mycologie médicale*, *24*(3), 193–204. https://doi.org/10.1016/j. mycmed.2014.02.009

Ismaiel, Ahmed A., & Ali, D. M. I. (2017). Antimicrobial properties of 6-pentyl-α-pyrone produced by endophytic strains of *Trichoderma koningii* and its effect on aflatoxin B1 production. *Biologia (Poland)*, *72*(12).1403-1415. https://doi. org/ 10.1515/biolog-2017-0173

Ismaiel, Ahmed A, El-, K. A., El-sherbeny, K. A., Abd, D. A., & Salem, E. (2022). Effect of Some Plant Extracts on the Growth and Mycotoxin Production of Three Toxigenic *Aspergillus* Strains Isolated from Raw Meat. *Middle East Journal of Applied Sciences*,12(2): 106–115. https:// doi.org/10.36632/mejas/2022.12. 2.12

Ismaiel, Ahmed A., Mohamed, H. H., & El-Sayed, M. T. (2023). Biodegradation of ochratoxin A by endophytic *Trichoderma koningii* strains. *World Journal of Microbiology and Biotechnology*, *39*(2), 1–13. Springer Netherlands. Retrieved from https://doi.org/ 10.1007/s11274-022-03491-2

Istiqomah, L., Damayanti, E., Arisnandhy, D., Setyabudi, F. M. C. S., & Anwar, M. (2019). Saccharomyces cerevisiae B18 as antifungal and aflatoxin binder in vitro. *AIP Conference Proceedings*, *2099*. https:// doi. org/10.1063/1.5098414

Joannis-Cassan, C., Tozlovanu, M., Hadjeba-Medjdoub, K., Ballet, N., & Pfohl-Leszkowicz, A. (2011). Binding of zearalenone, aflatoxin B1, and ochratoxin A by yeast-based products: A method for quantification of adsorption performance. *Journal of Food Protection*, *74*(7), 1175–1185. https://doi. org/ 10. 4315/ 0362-028X.JFP-11-023

Kanpiengjai, A., Mahawan, R., Lumyong, S., & Khanongnuch, C. (2016). A soil bacterium *Rhizobium borbori* and its potential for citrinin-degrading application. *Annals of Microbiology*, *66*(2), 807–816. https: //doi. org/10.1007/s13213-015-1167-1

Kong, C., Shin, S. Y., & Kim, B. G. (2014). Evaluation of mycotoxin sequestering agents for aflatoxin and deoxynivalenol: An in vitro approach. *SpringerPlus*, *3*(1). https: //doi. org/10. 1186/2193-1801-3-346

Lee, Y. K., El-Nezami, H., Haskard, C. A., Gratz, S., Puong, K. Y., Salminen, S., & Mykkänen, H. (2003). Kinetics of adsorption and desorption of aflatoxin B1 by viable and nonviable bacteria. *Journal of Food Protection*, *66*(3).426-430. https://doi.org/ 10.4315/0362-028X-66.3.426

Li, K., Cai, H., Luo, B., Duan, S., Yang, J., Zhang, N., He, Y., et al. (2025). Recent Progress of Mycotoxin in

Various Food Products—Human Exposure and Health Risk Assessment. *Foods*, *14*(5), 865. https://doi.org/10.3390/foods14050865

Ling, L., Li, Z., Jiao, Z., Zhang, X., Ma, W., Feng, J., Zhang, J., et al. (2019). Identification of Novel Endophytic Yeast Strains from Tangerine Peel. *Current Microbiology*, *76*(9).1066-1072. https: //doi. org/10. 1007/s00284-019-01721-9

Lv, Y. li, Zhang, F. sheng, Chen, J., Cui, J. long, Xing, Y. mei, Li, X. dong, & Guo, S. xing. (2010). Diversity and antimicrobial activity of endophytic fungi associated with the alpine plant *Saussurea involucrate*. *Biological and Pharmaceutical Bulletin*, *33*(8). https://doi.org/10.1248/bpb.33.1300

Malone, B. R., Humphrey, C. W., Romer, T. R., & Richard, J. L. (1998). One-Step Solid-Phase Extraction Cleanup and Fluorometric Analysis of Deoxynivalenol in Grains. *Journal of AOAC International*, *81*(2), 448–452. https://doi.org/10.1093/jaoac/81.2.448

Moubasher, A. H. (1993). Soil Fungi in Qatar and Other Arab Countries. Doha: The Centre of Scientific and Applied Research University of Qater,Doha.

Nasser, L. A. (2015). Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia. *Saudi Journal of Biological Sciences*, *22*(5).513-520. https://doi.org/10.1016/j.sjbs.2014.08.003

Ndiaye, S., Zhang, M., Fall, M., Ayessou, N. M., Zhang, Q., & Li, P. (2022). Current Review of Mycotoxin Biodegradation and Bioadsorption: Microorganisms, Mechanisms, and Main Important Applications. *Toxins*, *14*(11).729. https://doi.org/10.3390/toxins14110729

Okechukwu, V. O., Adelusi, O. A., Kappo, A. P., Njobeh, P. B., & Mamo, M. A. (2024). Aflatoxins: Occurrence, biosynthesis, mechanism of action and effects, conventional/ emerging detection techniques. *Food Chemistry*, *436*(April 2023), 137775. https://doi.org/10.1016/j.foodchem.2023.137775

Ostry, V., Malir, F., & Ruprich, J. (2013). Producers and important dietary sources of ochratoxin A and citrinin. *Toxins*, *5*(9). 1574-1586. https://doi.org/10.3390/toxins5091574

Panda, B. P., & Ali, M. (2012). Reduction of citrinin biosynthesis by fatty acids in *Monascus* fermented food. *World Mycotoxin Journal*, *5*(2). https://doi.org/10.3920/WMJ2011.1335

Peng, X., Wang, Y., Tang, L. J., Li, X. X., Xiao, Y. W., Zhang, Z. Bin, Yan, R. M., et al. (2018). Yeasts from Nanfeng mandarin plants: occurrence, diversity and capability to produce indole-3-acetic acid. *Biotechnology and Biotechnological Equipment*, *32* (6).1496-1506. https://doi.org/10.1080/13102818.2018.1487337

Pizzolitto, R. P., Bueno, D. J., Armando, M. R., Cavaglieri, L. R., Dalcero, A. M., & Salvano, M. A. (2011). Binding of aflatoxin B1 to lactic acid bacteria and *Saccharomyces cerevisiae* in vitro: a useful model to determine the most efficient microorganism. InTech-Open Access Publisher. http://dx.doi.org/ 10.5772/23717

Poloni, V., Dogi, C., Pereyra, C. M., Fernández Juri, M. G., Köhler, P., Rosa, C. A. R., Dalcero, A. M., et al. (2015). Potentiation of the effect of a commercial animal feed

additive mixed with different probiotic yeast strains on the adsorption of aflatoxin B1. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *32*(6). https://doi.org/10.1080/19440049.2015.1024761

Priyadarshini, E., & Tulpule, P. G. (1980). Effect of free fatty acids on aflatoxin production in a synthetic medium. *Food and Cosmetics Toxicology*, *18*(4).367-369. https://doi.org/10.1016/0015-6264 (80) 90191-1

Qiu, M., Wang, Y., Sun, L., Deng, Q., & Zhao, J. (2021). Fatty acids and oxylipins as antifungal and anti-mycotoxin agents in food: A review. In *Toxins* (Vol. 13, Issue 12).852. https://doi.org/10.3390/toxins13120852

Ren, X., Zhang, Q., Zhang, W., Mao, J., & Li, P. (2020). Control of Aflatoxigenic Molds by Antagonistic. *Toxins*, *12*(24), 1–21. https://doi.org/10.3390/toxins12010024

Sampaio Baptista, A., Horii, J., Antonia Calori-Domingues, M., Micotti Da Glória, E., Mastrodi Salgado, J., & Roberto Vizioli, M. (2004). The capacity of manno-oligo-saccharides, thermolysed yeast and active yeast to attenuate aflatoxicosis. *World Journal of Microbiology and Biotechnology*, *20*(5). https://doi.org/10.1023/B:WIBI.0000040397.48873.3b

Santin, E., Carlos Paulillo, A., Maiorka, A., Okada Nakaghi, L. S., Macari, M., da Silva, A. V. F., & Carlos Alessi, A. (2003). Evaluation of the efficacy of *Saccharomyces cerevisiae* cell wall to ameliorate the toxic effects of aflatoxin in broilers. *International Journal of Poultry Science*, *2*(5).341-344. https://doi.org/10.3923/ijps.2003.341.344

Sharma, D., Pramanik, A., & Agrawal, P. K. (2016). Evaluation of bioactive secondary metabolites from endophytic fungus Pestalotiopsis neglecta BAB-5510 isolated from leaves of *Cupressus torulosa* D.Don. *3Biotech*, *6*(2), 1–14. https://doi. org/ 10.1007/ s13205-016-0518-3

Shekhar, R., Raghavendra, V. B., & Rachitha, P. (2025). A comprehensive review of mycotoxins, their toxicity, and innovative detoxification methods. In Toxicology Reports (Vol. 14). 101952.Elsevier Inc. https:// doi. org/10.1016/j.toxrep.2025.101952.

Shetty, P. H., Hald, B., & Jespersen, L. (2007). Surface binding of aflatoxin B1 by *Saccharomyces cerevisiae* strains with potential decontaminating abilities in indigenous fermented foods. *International Journal of Food Microbiology*, *113*(1), 41–46. https://doi.org/10.1016/j.ijfoodmicro.2006.07.013

Snyder, L. (2008). Solvent selectivity in normal-phase TLC. *JPC-Journal of Planar Chromatography-Modern TLC*, *21*(5), 315–323. Akadémiai Kiadó.

Stahl, E. (1969). Apparatus and general techniques in TLC. *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52–86). Springer.

Sun, Y., Huang, K., Long, M., Yang, S., & Zhang, Y. (2022). An update on immunotoxicity and mechanisms of action of six environmental mycotoxins. *Food and Chemical Toxicology*, *163*, 112895. https://doi.org/10.1016/j.fct.2022.112895

Taheur, F. Ben, Fedhila, K., Chaieb, K., Kouidhi, B., Bakhrouf, A., & Abrunhosa, L. (2017). Adsorption of aflatoxin B1, zearalenone and ochratoxin A by microorganisms isolated from Kefir grains. *International Journal of Food*

Microbiology, *251*.PP,1-7. https://doi.org/10.1016/j.ijfoodmicro.2017.03.021

Taiwo, A. A. (2020). Evaluation of Microbiological Quality in Expired and Non-expired Dairy Canned Food Products Sold from Samaru Market, Zaria, Northwest Nigeria. *Journal of Clinical Immunology and Infectious Diseases* Volume 4 No.3

Tiwari, R. P., Mittal, V., Singh, G., Bhalla, T. C., Saini, S. S., & Vadehra, D. V. (1986). Effect of fatty acids on aflatoxin production by *Aspergillus parasiticus*. *Folia Microbiologica*, *31*(2). 120-123. https://doi.org/10.1007/BF02926829

Topcu, A., Bulat, T., Wishah, R., & BoyacI, I. H. (2010). Detoxification of aflatoxin B1 and patulin by *Enterococcus faecium* strains. *International Journal of Food Microbiology*, *139*(3).202-205. https://doi.org/10.1016/j.ijfoodmicro.2010.03.006

Ul Hassan, Z., Oufensou, S., Zeidan, R., Migheli, Q., & Jaoua, S. (2023). Microbial volatilome in food safety. Current status and perspectives in the biocontrol of mycotoxigenic fungi and their metabolites. *Biocontrol Science and Technology*, *33*(6), 499–538. https://doi.org/10.1080/09583157.2023.2205616

Walters, D. R., Walker, R. L., & Walker, K. C. (2003). Lauric acid exhibits antifungal activity against plant pathogenic fungi. *Journal of Phytopathology*, *151*(4).228-230. https://doi. org/10. 1046/ j.1439-0434.2003. 00713.x

Walters, D., Raynor, L., Mitchell, A., Walker, R., & Walker, K. (2004). Antifungal activities of four fatty acids against Plant Pathogenic fungi. *Mycopathologia*, *157* (1).87-90. https://doi.org/10. 1023 /B:MYCO.0000012222.68156.2c

Yan, P. S., Song, Y., Sakuno, E., Nakajima, H., Nakagawa, H., & Yabe, K. (2004). Cyclo(L-leucyl-L-prolyl) produced by *Achromobacter xylosoxidans* inhibits aflatoxin production by *Aspergillus parasiticus*. *Applied and Environmental Microbiology*, *70*(12), 7466–7473. https://doi.org/ 10.1128/AEM.70.12.7466-7473.2004

Yan, S., Liang, Y., Zhang, J., Chen, Z., & Liu, C. M. (2014). Autoxidated linolenic acid inhibits aflatoxin biosynthesis in *Aspergillus flavus* via oxylipin species. *Fungal Genetics and Biology*, *81*.pp.229-237. https://doi.org/10.1016/j. fgb. 2014.11.005

Yang, K., Geng, Q., Song, F., He, X., Hu, T., Wang, S., & Tian, J. (2020). Transcriptome sequencing revealed an inhibitory mechanism of *Aspergillus flavus* asexual development and aflatoxin metabolism by soy-fermenting non-aflatoxigenic *Aspergillus*. *International Journal of Molecular Sciences*, *21*(19), 1–17. https://doi.org/10.3390/ijms21196994

Zahra, N., Jamil, N., Ahmad, S. R., Saeed, M. K., Kalim, I., & Sheikh, A. (2019). A review of mycotoxin types, occurrence, toxicity, detection methods and control. *Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences*, *62*(3), 206–218. https://doi.org/10.52763/pjsir.biol.sci.62.3.2019.206.218

Zargar, S., & Wani, T. A. (2023). Food Toxicity of Mycotoxin Citrinin and Molecular Mechanisms of Its Potential Toxicity Effects through the Implicated Targets Predicted by Computer-Aided Multi-dimensional Data Analysis. *Life*,

13(4). https://doi.org/10.3390/life13040880

Zhang, W., Chang, X., Han, Y., Li, T., Dou, J., Du, W., Wu, W., et al. (2025). Biodegradation of aflatoxin B1 by a novel mined aldo–keto reductase from *Meyerozyma guilliermondii* AF01. *Biological Control*, vol. *200* pp.105676 https://doi.org/10.1016/j.biocontrol.2024.105676

Zhang, W., Sun, C., Wang, W., & Zhang, Z. (2024). Bioremediation of Aflatoxin B1 by *Meyerozyma guilliermondii* AF01 in Peanut Meal via Solid-State Fermentation. *Toxins*, *16*(7).305. https://doi. org/10.3390/toxins16070305

Zhang, W., Wang, J., Dou, J., Li, T., Liu, H., Chang, X., Qian, S., et al. (2023). A novel investigated method for decoupling adsorption and degradation effect on AFB1 based on isotope tracing and NMR analysis. *Food Chemistry*, *405* pp.134978 https://doi.org/10.1016/j.foodchem.2022.134978

Zheng, M. Z., Richard, J. L., & Binder, J. (2006). A review of rapid methods for the analysis of mycotoxins. In *Mycopathologia*, Vol. 161, (5). 261-273. https://doi.org/10.1007/s11046-006-0215-6

Zhu, Y., Hassan, Y. I., Lepp, D., Shao, S., & Zhou, T. (2017). Strategies and methodologies for developing microbial detoxification systems to mitigate mycotoxins. *Toxins*, *9*(4).130. https://doi.org/10.3390/toxins9040130