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Heat stability of *Trichoderma asperelloides* SKRU-01 culture filtrates: Potential applications for controlling fungal spoilage and AFB_1 production in peanuts

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ABSTRACT

This study aimed to examine the heat stability of culture filtrates of *Trichoderma asperelloides* SKRU-01 (culture filtrates SKRU-01) over a temperatures range (40–121 °C) and the effects on the antifungal activity against two aflatoxin-producing strains (*Aspergillus parasiticus* TISTR 3276 and *A. flavus* PSRDC-4), aflatoxin B_1 (AFB_1) degradation, and the role in mycotoxin control in peanuts. The impact of SKRU-01 culture age (2–12 day-old) on both pathogenic strains revealed that the culture age of 6–12 day-old cultures exhibited no significant difference ($p > 0.05$) of growth inhibition for strain TISTR 3276 (81.89–82.28 %) and 4–12 day-old cultures for strain PSRDC-4 (74.87–79.06 %). The heat-treated temperatures from 40 °C to 121 °C caused no significant ($p > 0.05$) reduction of mycelial growth for strain TISTR 3276 (82.61 % to 79.13 %) but significant ($p < 0.05$) deduction for strain PSRDC-4 (75.15 % to 59.17 %). Heat treatment of the culture filtrates SKRU-01 at 60–121 °C caused the reduction on spore germination inhibition (from about 68 % to 58.16 % for strain TISTR 3276 and 51.11 % for strain PSRDC-4). These results indicate that strain TISTR 3276 exhibited greater susceptibility to culture filtrates SKRU-01 compared to strain PSRDC-4. Furthermore, the culture filtrates SKRU-01 exhibited remarkable thermal stability at 121 °C, degrading AFB_1 to 63.91 %. Application of heat-stable culture filtrates SKRU-01 in peanuts demonstrated that the reduction in fungal population and AFB_1 production of both pathogenic strains depended significantly ($p < 0.05$) on the level of heat treatment. The non-treated and 40 °C treated culture filtrates SKRU-01 could reduce AFB_1 production to lower than the Standard Aflatoxin Limitation (<20 μg/kg), ensuring food safety and mitigating the health risks associated with aflatoxin exposure.

1. Introduction

Aspergillus flavus and *A. parasiticus* are well-known for their aflatoxin-producing capabilities, particularly in oil-rich crops such as peanuts, maize, rice, and cottonseed, as well as in processed products during storage (Ma et al., 2021; Gong et al., 2023). During both pre- and post-harvest stages, these fungi are capable of producing highly toxic secondary metabolites known as aflatoxins B_1 (AFB_1), which the International Agency for Research on Cancer (IARC) evaluated through both epidemiological and laboratory studies. The IARC categorized aflatoxins as carcinogenic (Group 1) (World Health Organization, 1993; Ma et al., 2021) and potentially carcinogenic to humans (Group 2B) (World Health Organization, 1993). As it poses a grave hazard to human health, the presence of aflatoxins in crops and agricultural products is a significant global health concern (World Health Organization, 1993).

Numerous strategies have been employed to manage aflatoxin-producing fungi (Chiuraise et al., 2015; Ren et al., 2022) and to deactivate and detoxify aflatoxins in crops, both before and after harvest (Spadaro and Garibaldi, 2017). These strategies encompass a range of approaches, including physical, chemical, and biological methods (Siciliano et al., 2016). To mitigate the risk of excessive fungicide usage, there is a growing interest in exploring alternative methods for

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controlling pathogenic and mycotoxigenic fungi, with a particular focus on biocontrol agents. The utilization of microorganisms as biological control agents has witnessed significant growth in recent decades (Siahmoshteh et al., 2017).

Several antagonists were applied for this aim, but atoxigenic fungi and bacterial strains are commonly used (Bandyopadhyay et al., 2016). The ideal antagonist should have certain characteristics, such as genetic stability, efficacy at low concentrations against a wide range of pathogens on fruit products, simple nutritional requirements, survival in adverse environmental conditions, growth on cheap substrates in fermenters, lack of pathogenicity for the host plant and lack of toxigenicity to humans, resistance to the most frequently used pesticides and compatibility with other chemical and physical treatments (Spadaro and Droby, 2016).

In recent years, antagonists have garnered significant attention due to their diverse biological activities, including their capacity to generate novel chemical compounds of high commercial value (Amador et al., 2003). The use of several species of antagonistic microorganisms, such as *Streptomyces* species (Campos-Avelar et al., 2021; Boukaew et al., 2023a, 2023b, 2023c), *Bacillus* species (Li et al., 2022; Yuan et al., 2023), *Pseudomonas* species (Gomaa et al., 2019; Gong et al., 2022), and *Trichoderma* species (Ren et al., 2022; Boukaew et al., 2023d; Madbouly et al., 2023), has gained significant attention in minimizing the impact of mycotoxins. Beneficial microorganisms are increasingly being used as biocontrol agents due to their high efficiency, specificity, and cost-effectiveness (Mason et al., 2023).

Fungi from the *Trichoderma* genus have gained substantial recognition as potent biocontrol agents against plant-pathogenic fungi (Ayyandurai et al., 2023; Behiry et al., 2023), including *Aspergillus* species (Dini et al., 2022; Ren et al., 2022; Madbouly et al., 2023). Notably, *T. hamatum* exhibits a distinctive ability to grow toward host hyphae and, upon contact, form coils and appressoria-like structures that facilitate penetration (Chet and Baker, 1981). Despite documented instances of parasitic behavior in *Trichoderma* spp., investigations within *A. flavus-Trichoderma* spp. interactions have revealed no evident parasitic activity (Verma et al., 2007; Ren et al., 2022). *Trichoderma* species employed as antagonists in biological control against other microorganisms have been proposed to inhibit growth through three primary mechanisms: parasitism (nutrient extraction from the host), competition (for resources and space), and antibiosis (production of inhibitory metabolites or antibiotics) (Whipps and Lumsden, 1991; Howell, 2003). While one mechanism may dominate, it's important to recognize that this does not preclude the possibility of the other two mechanisms also contributing to their antagonistic behavior. This genus is well-documented for its effectiveness as biocontrol agents, often relying on the production of secondary metabolites with antibiotic activity (Vinale et al., 2008; Ren et al., 2022). Over the years, researchers have identified >1000 secondary metabolites from various *Trichoderma* spp. (Hermosa et al., 2014), some of which have found successful applications in controlling plant diseases (Calistru et al., 1997; Branà et al., 2017), including those produced by *T. asperelloides* (Boukaew et al., 2023d). However, despite the proficiency of several *Trichoderma* species, including *T. harzianum*, *T. reesei*, *T. koningii*, and *T. asperellum*, in degrading hydrocarbon compounds such as polycyclic aromatic hydrocarbons (PAHs), crude oil, and resins (Zafra and Cortés-Espinosa, 2015), there is a notable lack of reports on the ability of *Trichoderma* spp. to biodegrade small molecule contaminants, particularly aflatoxins (Palonen et al., 2004; Pribowo et al., 2012; Hackbart et al., 2014; Yue et al., 2022). The capacity of antifungal agents produced by *T. asperelloides* SKRU-01 for aflatoxin degradation remains largely unexplored. Hence, this study aims to evaluate the effectiveness of bioactive compounds in culture filtrates SKRU-01 for degrading AFB_1, offering potential applications in reducing aflatoxin contamination in stored grains.

Our recent study (Boukaew et al., 2023d) demonstrated that *T. asperelloides* SKRU-01 effectively inhibited aflatoxigenic *A. flavus* and *A. parasiticus*, reducing food and feed spoilage in peanuts through the production of antifungal agents. However, the potential of these heat-stable antifungal agents for AFB_1 degradation and mycotoxin production control in peanuts remains unexplored. These heat-stable agents offer a promising strategy due to their resilience to high temperatures, making them effective even in thermally demanding environments like food production and storage. This study aims to investigate the capability of heat-stable antifungal agents produced by *T. asperelloides* SKRU-01 in controlling AFB_1 degradation and mycotoxin production in peanuts. This research aims to elucidate how *Trichoderma*-based antifungal agents, known for producing antibiotics, can effectively combat aflatoxin contamination in thermally challenging conditions associated with food production and storage. Our objective is to update knowledge about the efficacy of these agents in preventing aflatoxigenic *A. flavus* and *A. parasiticus*-related food and feed spoilage while exploring their potential as a safe and effective approach to mitigate mycotoxin contamination in peanuts.

2. Material and methods

2.1. Culture media and microorganisms

Strain SKRU-01 was isolated from loam soil samples and identified to be *Trichoderma asperelloides* (Boukaew et al., 2023d). The strain was cultivated on potato dextrose agar (PDA) (39 g/L, Difco Laboratory) at 30 °C for 7 days. Spores were extracted from the surface of the agar and suspended in a 5 mL solution of sterile distilled water and 0.1 % (v/v) Tween 80 (Sigma-Aldrich). After passing the spore suspensions through sterile cheesecloth to remove any lingering mycelia, the spore count was determined using a hemocytometer. The final concentration of 10^5 spores/mL was attained by adjusting the concentration with sterile water to meet the experimental requirements.

A. parasiticus TISTR 3276 and *A. flavus* PSRDC-4 were previously identified as high aflatoxin producers (Boukaew et al., 2020a). Fungi were cultured on PDA plates at 30 °C for seven days and spores were collected using the same technique.

2.2. Assessing the impact of culture age on antifungal activity of strain SKRU-01 against both mycotoxin-producing strains

A spore suspension of strain SKRU-01, containing 10^5 spores/mL, was added to 100 mL of potato dextrose broth (PDB) (24 g/L; Difco Laboratory) medium in 250 mL flasks with the pH adjusted to 7.0 using 5 M NaOH before autoclaving. The flasks were incubated at 30 °C on a rotary shaker at 150 rpm for 0, 2, 4, 6, 8, 10, and 12 d. After incubation, the mycelial mats were filtered from the culture broth using a Whatman No. 1 filter paper (125 mm diameter; Whatman™). The resulting filtrate was then passed through a 0.45 μm Millipore membrane (47 mm diameter; Sartorius®) to obtain the culture filtrates of SKRU-01.

To investigate the anti-mycotoxin-producing fungal activity, 10 mL of molten sterile PDA was mixed with the culture filtrate of strain SKRU-01 at different culture ages, and poured onto a 9 cm diameter culture plate. As a control, 10 mL of melted sterile PDA was mixed with PDB medium (without culture filtrates SKRU-01) at an equivalent amount. Then, a 5 μL suspension containing 10^5 spores/mL of each mycotoxin-producing spore was dropped onto the center of the test agar plates. The experiment was replicated three times for each treatment. The colonies of each mycotoxin-producing fungus were measured for diameter after incubating for 5 days at 30 °C. The percentage inhibition of hyphal growth was then determined using the formula: Percentage inhibition (%) = [(Dc × Dt)/Dc] × 100, where Dc represents the mycelial growth of the fungus on the control plate, and Dt represents the mycelial growth of the fungus on the test plate.

2.3. Effect of culture filtrates SKRU-01 treated at various temperatures on mycelial growth inhibition, spore germination, and AFB_1 degradation

2.3.1. Antifungal activity against mycotoxin-producing strains (mycelial growth inhibition)

After preparing a culture filtrate of SKRU-01 (as previously described), the resulting culture filtrate with a pH of 6.7 was divided into two equal portions. One of the samples served as a control, while the other was incubated at varying temperatures (40 °C, 60 °C, 80 °C, and 100 °C) for 30 min in a water bath and at 121 °C for 15 min by autoclaving. The antifungal activity of both the heated and non-heated culture filtrates SKRU-01 was then evaluated using the bioassay procedure described in Section 2.2. Each treatment was administered three times.

2.3.2. Spore germination inhibition

To assess the antifungal efficacy of both heated (40–121 °C) and non-heated culture filtrates SKRU-01 against spore germination of both pathogenic strains, a 50 μL suspension containing 10^5 spores/mL of each mycotoxin-producing spore was mixed with 50 μL of each culture filtrates. As a control, sterile distilled water was used. The mixtures were incubated for 12 h at 30 °C on a rotary shaker at 150 rpm. Subsequently, 50 μL of the mixed culture was dropped onto PDA Petri dishes and spread evenly. After 24 h, the fungal colonies were counted, and the total number of live germinated spores per microliter was determined to calculate the percentage of spore germination (Li et al., 2013). Each treatment was performed in triplicate.

2.3.3. AFB_1 degradation

The ability of both heated (40–121 °C) and non-heated culture filtrates SKRU-01 to degrade commercial AFB_1 (Siam Inter Quality Co., Ltd., Thailand) was evaluated in 2-mL Eppendorf tubes with a final volume of 1.0 mL, using a modified version of the method developed by Teniola et al. (2005). Briefly, 50 μL of an AFB_1 stock solution containing an initial concentration of 40.0 μg/mL was combined with 950 μL of each treatment. According to Xia et al. (2017), the mixture was then incubated in the dark at 30 °C for 96 h without stirring. As a control, sterile distilled water with AFB_1 was used. After treatment, samples were extracted with chloroform according to the method described by Tosch et al. (1984), and the AFB_1 concentration was determined by indirect competitive ELISA using a ScreenEZ® Aflatoxin ELISA test kit (Siam Inter Quality Co., Ltd., Thailand), as detailed by Boukaew et al. (2020b). The absorbance at 450 nm (OD450) was measured using a microplate reader (M965 + MetertechInc., Taiwan), and the AFB_1 concentration was estimated using a Stat Fax Reader Model 321 by subtracting OD450 from OD450. The experiment was conducted in triplicate for each treatment. The percentage was calculated using the formula: [(Control-treatment)/Control] × 100 (Branà et al., 2017), where the control concentration of AFB_1 was treated with dimethyl sulfoxide (DMSO; Sigma-Aldrich) and the treatment concentration was treated with culture filtrates SKRU-01.

2.4. Kinetics of AFB_1 degradation by culture filtrates SKRU-01

Using a modified version of the method devised by Teniola et al. (2005), the degradation kinetics of AFB_1 by culture filtrates SKRU-01 were examined. The experiment was conducted in 2 mL Eppendorf tubes with a final volume of 1.0 mL. To initiate the experiment, 50 μL of a 40.0 μg/mL AFB_1 stock solution was mixed with 950 μL of each treatment. The mixtures were then incubated without shaking at 30 °C in the dark for different durations (0, 12, 24, 36, 48, 60, 72, 84, and 96 h) as described by Xia et al. (2017). A control consisting of sterile distilled water plus AFB_1 was also used. The analysis of AFB_1 degradation in the samples was performed using the method previously mentioned. The experiment was conducted in triplicate for each treatment.

2.5. Biocontrol efficacy of heat-stable culture filtrates SKRU-01 for controlling two mycotoxin-producing strains in peanuts

2.5.1. Peanut preparation

Raw peanut kernels were purchased from a nearby grocery store and stored at −20 °C until use. Before testing, the kernels were surface-sterilized by immersing them in a 3 % (v/v) sodium hypochlorite solution for 3 min and then washing them multiple times with sterile distilled water, according to Abd-Alla's (2005) method. Following this, they were autoclaved at 121 °C for 15 min.

2.5.2. HPLC-FLD methods validation

The analytical method of HPLC-FLD for mycotoxins followed the procedure outlined by Choochuay et al. (2018). Validation included assessing parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), and precision. We referred to Thailand's Animal Feed Quality Control Act B.E. 2525, which specifies maximum permitted levels (MPLs) of mycotoxins in animal feed, as the standard for our analysis (Choochuay et al., 2018). The maximum fortified concentration for the samples was set at 100 μg/kg in accordance with these MPLs.

An external standardization approach was used to evaluate linearity. A series of AFB_1 standard solutions with six distinct concentrations ranging from 5 to 100 μg/kg (5, 10, 20, 40, 60, and 100 μg/kg) were prepared. Then, calibration curves for the matrices were created by plotting the peak areas, which represented the analytical signal response (y), against the respective AFB_1 concentrations (x). This permitted the development of analytical curves for precise measurement. The specificity of the method was evaluated by comparing the retention periods of blank sample matrices to those of samples containing 100 μg/kg AFB_1. This analysis ensured that the retention time of the analyte of interest remained unaffected and unimpeded (Choochuay et al., 2018).

Limits of detection (LOD) and quantitation (LOQ) were evaluated to determine the method's sensitivity. The LOD was determined as the lowest concentration of AFB_1 that produced a signal response that was three times greater than the average baseline noise observed in 10 independent blank samples of each matrix (S/N: 3:1). Alternatively, the LOQ was defined as the AFB_1 signal response that exceeded the average baseline noise by a factor of ten in ten independent blank samples of each matrix (S/N: 10:1) (Xie et al., 2018). These measures help determine the lowest detectable and quantifiable levels of AFB_1 in the samples, ensuring the sensitivity and precision of the method.

Spiking blank sample matrices with AFB_1 standard solutions at three discrete concentration levels (equivalent to 20, 40, and 100 μg/kg) was used to evaluate the test's precision. Six replicates of each concentration in each matrix were prepared. This allowed for the evaluation of the method's accuracy by determining how close the measured values were to the genuine values (Choochuay et al., 2018). As relative standard deviations (RSDs), intraday repeatability (RSD_r) and interday reproducibility (RSD_R) were calculated to evaluate precision. At the three concentration levels (20, 40, and 100 μg/kg), standard solutions of AFB_1 were added to blank samples, and six replicates were prepared for each concentration in each matrix. By analyzing the RSDs, the precision and consistency of the procedure were determined, providing valuable insight into its consistency over time and within the same day (Choochuay et al., 2018).

2.5.3. Effect of heat-stable bioactive compounds in culture filtrates SKRU-01 on peanuts inoculated with two mycotoxin-producing fungal spores

According to Yang and Chang (2010), peanut samples were treated with heated (40–121 °C) and non-heated culture filtrates SKRU-01 for 8 h at 30 °C. As a control, peanuts soaked in sterile distilled water were used. After treatment, the peanuts were dried in a laminar airflow for 30 min and then transferred to 140 mm Petri dishes. A spore suspension of the two aflatoxigenic fungi strains was prepared at a concentration of 10^5 spores/mL. Approximately 1 mL of the suspension was directly inoculated onto each peanut (30 peanuts in total/treatment), which

were then incubated at 30 °C for 5 d. After 5 d, the peanuts inoculated with the two strains of fungi were divided into two equal batches: one batch was used to determine mold density, and the other was used to measure AFB_1 production.

To determine the density of mold in each peanut sample, 5 g of the sample was mixed with 45 mL of a saline solution containing 0.85 % NaCl and agitated on a rotary shaker at 200 rpm for 2 h at 30 °C. The resulting suspension was then serially diluted two-fold in saline solution, and 0.1 mL of the diluted suspension was spread onto PDA plates and incubated at 30 °C for 48 h. The mold density was determined by calculating the logarithm of spores per gram of peanuts. The percentage reduction in spores was calculated as previously described. All treatments were performed in triplicate.

The following procedures were performed to determine the concentration of aflatoxin AFB_1. First, 10 g of pulverized peanut powder was combined with 25 mL of 70 % aqueous methanol and homogenized for 3 min using a laboratory homogenizer. Afterward, the mixture was filtered with Whatman No. 1 filter paper. The concentration of AFB_1 was determined using High-performance liquid chromatography with fluorescence detection (HPLC-FLD; Agilent Technologies, Wilmington, DE, USA), as described by Choochuay et al. (2018). The experiment was conducted in triplicate for each treatment.

2.6. Statistical analysis

The experimental procedures were repeated three times, and the collected data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp; IBM SPSS Statistics for Windows, version 26.0, Armonk, NY). The data were then analyzed using variance analysis (ANOVA). When deemed necessary, means were compared using Tukey's HSD (Truly Significant Difference) test, with a significance level of $p < 0.05$.

3. Results

3.1. Assessing the impact of culture age on antifungal activity of strain SKRU-01 against both mycotoxin-producing strains

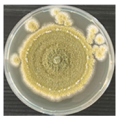
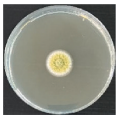
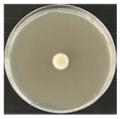
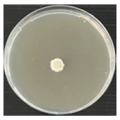
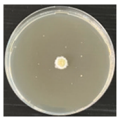
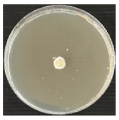
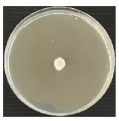
Tables 1 and 2 display the in vitro susceptibilities of aflatoxin-producing *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 (10^5 spores/mL on PDA plates) when treated with various culture ages (0–12 d) of strain SKRU-01. As the culture age of strain SKRU-01 increased, the mycelial growth of both pathogenic strains decreased significantly ($p < 0.05$), to 81.89 % for 6 d culture age of strain TISTR 3276 (Tables 1) and 74.87 % for 4 d culture age of strain PSRDC-4 (Table 2). It should be notes that the fungal growth of both pathogenic strains was inhibited by 50 % using 2 d culture age.

3.2. Effect of culture filtrates SKRU-01 treated at various temperatures on mycelial growth inhibition, spore germination, and AFB_1 degradation

The influence of incubation temperature (40–121 °C) on the culture filtrate SKRU-01 against the mycelial growth inhibition of aflatoxin-producing *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 is depicted in Tables 3 and 4, respectively. The mycelial growth of both pathogenic strains was significantly reduced ($p < 0.05$) following treatment with either heated or non-heated culture filtrates SKRU-01, as shown in Tables 3 and 4. The heat-treated temperatures from 40 °C to 121 °C caused no significant ($p > 0.05$) reduction of mycelial growth for strain TISTR 3276 (82.61 % to 79.13 %) but significant ($p < 0.05$) deduction for strain PSRDC-4 (75.15 % to 59.17 %). The non-heated culture filtrates SKRU-01 were the most effective against both strains, inhibiting strain TISTR 3276 and strain PSRDC-4 by 83.34 % and 79.88 %, respectively.

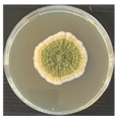
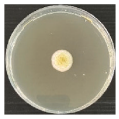
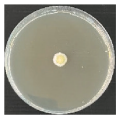
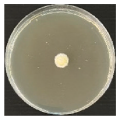
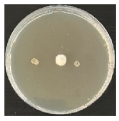
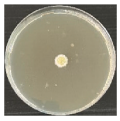
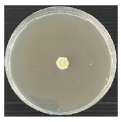
Fig. 1 displays the potent antifungal activity of culture filtrates SKRU-01 against spore germination of aflatoxin-producing strains TISTR 3276 and PSRDC-4 at various incubation temperatures (40–121 °C) for heated treatments. As shown in Fig. 1, treatment with both heated and non-heated culture filtrates SKRU-01 significantly ($p < 0.05$) decreased spore germination inhibition of both pathogenic strains (68.24 % to 58.16 % for strain TISTR 3276 and 68.06 % to 51.11 % for strain PSRDC-4). Heat treatment of the culture filtrates SKRU-01 at 60–121 °C caused the reduction on spore germination inhibition (from about 68 % to 58.16 % for TISTR 3276 and 51.11 % for PSRDC-4). These results indicated that strain TISTR 3276 exhibited greater susceptibility to culture filtrates SKRU-01 compared to strain PSRDC-4. The non-heated treatment significantly inhibited TISTR 3276 and PSRDC-4 strains spore germination by 68.72 % and 68.18 %, respectively. Nonetheless, there were no statistically significant ($p > 0.05$) disparities in inhibition when compared to the 40 °C treatment, which showed 69.27 % and 68.75 % inhibition, respectively. In addition, there were no statistically significant ($p > 0.05$) differences in spore germination inhibition among the 80 °C, 100 °C, and 121 °C heat protocols for each aflatoxin-producing

Table 1
The relationship between percentages of hyphal inhibition and culture age derived from strain SKRU-01 on the growth of *A. parasiticus* TISTR 3276 after an incubation at 30 °C for 5 d.

Culture age (d)	Radial growth (cm)	Inhibition of Radial growth (%)	Colony morphology
0 (Control)	$6.35^{a} \pm 0.13$	–	
2	$2.38^{b} \pm 0.19$	62.60 ± 1.89	
4	$1.45^{c} \pm 0.06$	77.17 ± 0.58	
6	$1.15^{d} \pm 0.06$	81.89 ± 0.57	
8	$1.18^{d} \pm 0.05$	81.50 ± 0.50	
10	$1.13^{d} \pm 0.05$	82.28 ± 0.50	
12	$1.10^{d} \pm 0.00$	82.68 ± 0.00	

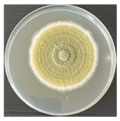
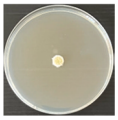
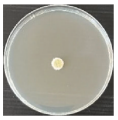
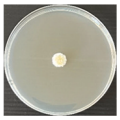
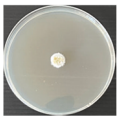
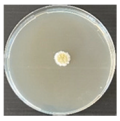
Note: Reported values represent means ± standard deviation from three replicates. Data followed by the same letter within each column show no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$).

Table 2
The relationship between percentages of hyphal inhibition and culture age derived from strain SKRU-01 on the growth of *A. flavus* PSRDC-4 after an incubation at 30 °C for 5 d.

Culture age (d)	Radial growth (cm)	Inhibition of Radial growth (%)	Colony morphology
0 (Control)	4.78^{a} ± 0.30	–	
2	2.00^{b} ± 0.00	58.12 ± 0.00	
4	1.20^{c} ± 0.08	74.87 ± 0.86	
6	1.15^{c} ± 0.13	75.92 ± 1.29	
8	1.10^{c} ± 0.08	76.96 ± 0.82	
10	1.03^{c} ± 0.05	78.53 ± 0.51	
12	1.00^{c} ± 0.00	79.06 ± 0.00	

Note: Reported values represent means ± standard deviation from three replicates. Data followed by the same letter within each column show no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$).

Table 3
Effect of culture filtrates SKRU-01 treated at various temperatures (40–121 °C) on the growth of *A. parasiticus* TISTR 3276 after incubation at 30 °C for 5 d.

Heat treatments	Radial growth (cm)	Mycelial growth inhibition (%)	Colony morphology
Control	5.75^{a} ± 0.50	–	
Non-treated	0.95^{b} ± 0.13	83.34 ± 1.29	
40 °C	1.00^{b} ± 0.08	82.61 ± 0.81	
60 °C	1.20^{b} ± 0.08	79.13 ± 0.86	
80 °C	1.40^{b} ± 0.08	75.65 ± 0.92	
100 °C	1.25^{b} ± 0.10	78.26 ± 1.00	
121 °C	1.20^{b} ± 0.08	79.13 ± 0.81	

Note: Reported values represent means ± standard deviation from three replicates. The control used sterile distilled water. Data followed by the same letter within each column show no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$).

strain, all of which demonstrated >50 % inhibition.

The effect of temperature (40–121 °C) on the ability of culture filtrates SKRU-01 to degrade AFB_1 after 96 h incubation at 30 °C is depicted in Fig. 2. Fig. 2A demonstrates that both heated and unheated (non-treated) culture filtrates SKRU-01 exhibited highly significant ($p < 0.05$) reductions in the AFB_1 concentration (40 μg/mL). After 96 h of incubation, the non-heated culture filtrates SKRU-01 degraded approximately 86.41 % of AFB_1, which was not significantly ($p > 0.05$) different from the 40 °C treated culture filtrate (85.99 %). Nevertheless, after 15 min of autoclaving at 121 °C, the AFB_1 degradation activity of culture filtrates SKRU-01 was reduced to 63.91 % (Fig. 2B).

3.3. Kinetics of AFB_1 degradation by culture filtrates SKRU-01

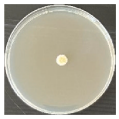
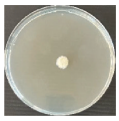
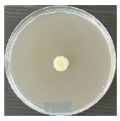
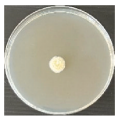
The ability of culture filtrates SKRU-01 to degrade AFB_1 is depicted in Fig. 3. AFB_1 degradation began at 24 h treatment (18.93 %), increased markedly at 36 h (30.57 %), and peaked at 72 h (82.73 %) with no significantly difference ($p > 0.05$) from treatment at both 84 h and 96 h. Therefore the optimum treatment time to degrade AFB_1 was 72 h, or 24 h reduction from the previous experiment (Section 3.2). In contrast, the concentration of AFB_1 in the control group remained relatively stable throughout the duration of incubation.

3.4. Biocontrol efficacy of heat-stable bioactive compounds in culture filtrates SKRU-01 for controlling two mycotoxin-producing strains in peanuts

3.4.1. HPLC-FLD method validation results

The HPLC-FLD method underwent validation by analyzing AFB_1 production (Table 5), with the validation parameters including the linear range, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. The results demonstrated that the LOD and LOQ

Table 4
Effect of culture filtrates SKRU-01 treated at various temperatures (40–121 °C) on the growth of *A. flavus* PSRDC-4 after incubation at 30 °C for 5 d.

Heat treatments	Radial growth (cm)	Mycelial growth inhibition (%)	
Control	4.23^{a} ± 0.22	–	
Non-treated	0.85^{f} ± 0.06	79.88 ± 0.57	
40 °C	1.05^{ef} ± 0.06	75.15 ± 0.74	
60 °C	1.13^{de} ± 0.05	73.37 ± 0.57	
80 °C	1.33^{cd} ± 0.05	68.64 ± 0.95	
100 °C	1.45^{c} ± 0.06	65.68 ± 0.57	
121 °C	1.73^{b} ± 0.21	59.17 ± 2.11	

Note: Reported values represent means ± standard deviation from three replicates. The control used sterile distilled water. Data followed by the same letter within each column show no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$).

were determined to be 0.9 μg/kg and 1.2 μg/kg, respectively. The correlation coefficients (R^2) exceeded 0.9995, indicating excellent linearity. Regarding precision, both intra-day and inter-day measurements were performed, yielding precision values of 1.02–5.10 % and 1.68–6.58 %, respectively. These outcomes highlight the robustness and suitability of the method for detecting AFB_1 production in peanuts. Notably, the recovery rate fell within the range of 92.89 % to 106.73 %, further confirming the accuracy of the method.

3.4.2. Efficacy of heat-stable bioactive compounds in culture filtrates SKRU-01 for controlling two mycotoxin-producing strains in peanuts

The effectiveness of both heated (40–121 °C) and non-heated culture filtrates SKRU-01 in preventing the growth of both strains, TISTR 3276 (Fig. 4A), and PSRDC-4 (Fig. 4B), on peanuts was investigated (Fig. 4, Tables 6). The control peanuts exhibited abundant mold growth and dark green spores, whereas those treated with both heated (40–121 °C) and non-heated culture filtrates SKRU-01 showed varying degrees of growth inhibition. The reduction in fungal population (spore reduction) and AFB_1 production of both pathogenic strains was significantly ($p < 0.05$) dependent on the heated and non-heated treatment of culture filtrates SKRU-01. After 5 d incubation, the control peanuts had a high fungal population (viable spores) and AFB_1 production: 3.87 log spores/g and 148.57 μg/kg for the strain TISTR 3276 (Table 6A). Non-heated culture filtrates SKRU-01 significantly ($p < 0.05$) reduced the fungal population (by 1.35 log spores/g) and aflatoxin production (by 17.64 μg/kg) in peanuts inoculated with the strain TISTR 3276, which was higher than the reductions achieved with the heated treatments (60–121 °C). However, increasing the heat treatments (40 to 121 °C) resulted in a decrease in spore reduction from 64.08 % to 45.22 %.

For the strain PSRDC-4, both control peanuts and peanuts treated with non-heated culture filtrates SKRU-01 showed similar growth and AFB_1 production (1.57 log spores/g and 19.30 μg/kg, respectively). However, different heated (40–121 °C) culture filtrates SKRU-01 treatments resulted in a decrease in spore reduction from 60.92 % to 39.56 % and an increase in total AFB_1 production from 20.01 μg/kg to 67.11 μg/kg (Table 6B).

4. Discussion

This study sought to evaluate the thermal stability of culture filtrates SKRU-01 in order to determine their potential as a biocontrol agent for mitigating *Aspergillus* species infestation in peanuts and promoting aflatoxin B_1 (AFB_1) degradation. The results demonstrated that the thermal stability of culture filtrates SKRU-01 exhibited significant ($p < 0.05$) efficacy in controlling the strains TISTR 3276 and PSRDC-4, as well as robust AFB_1 degradation.

The study's findings highlight the significance of culture age in determining the antagonistic effects of culture filtrates SKRU-01 against the examined pathogenic strains (TISTR 3276 and PSRDC-4). Our research revealed varying inhibitory effects on the radial mycelial growth of both pathogenic strains over time. Notably, the highest level of antifungal activity was observed in the 12-day-old culture, coinciding with the maximum incubation period examined in a previous study by Zafar et al. (2013) involving *Trichoderma* species. Nevertheless, its highest growth inhibition of the strain TISTR 3276 (82.68 %) was not significantly difference ($p > 0.05$) from those of 6–10 day-old culture (81.89–82.28 %). This means the optimum culture age of the strain TISTR 3276 was only 6-day-old. In similar pattern, the optimum culture age of the strain PSRDC-4 was 4-day-old with no significantly difference ($p > 0.05$) in the growth inhibition (74.87–79.06 %). While our findings offer valuable insights into short-term antifungal efficacy, it's essential to recognize the potential for dynamic changes in extended temporal studies, which necessitate further investigation. The observed time-dependent variation in antifungal effectiveness suggests that the metabolic activity and production of bioactive compounds in the culture of *T. asperelloides* SKRU-01 changed as it aged. As the culture matures, the concentration of bioactive substances responsible for inhibiting the growth of the pathogenic strains increased, leading to higher antifungal potential. These findings have significant implications for the practical application of *T. asperelloides* SKRU-01 as a biocontrol agent against *Aspergillus* species. To achieve the maximum antifungal effect, it is recommended to use cultures that are 4 days old for *A. flavus* and 6 days old for *A. parasiticus*, respectively. By considering culture age as a crucial factor in biocontrol efficacy, it becomes possible to optimize the application of this strain for controlling *Aspergillus*-related pathogens effectively. This knowledge can aid in developing more targeted and efficient strategies to combat *Aspergillus* infections in agricultural and industrial settings.

The results of this study demonstrated the inhibitory effects of culture filtrates SKRU-01 on fungal growth and spore germination. Interestingly, non-treated culture filtrates SKRU-01 exhibited slightly stronger inhibition compared to those subjected to heat treatment and

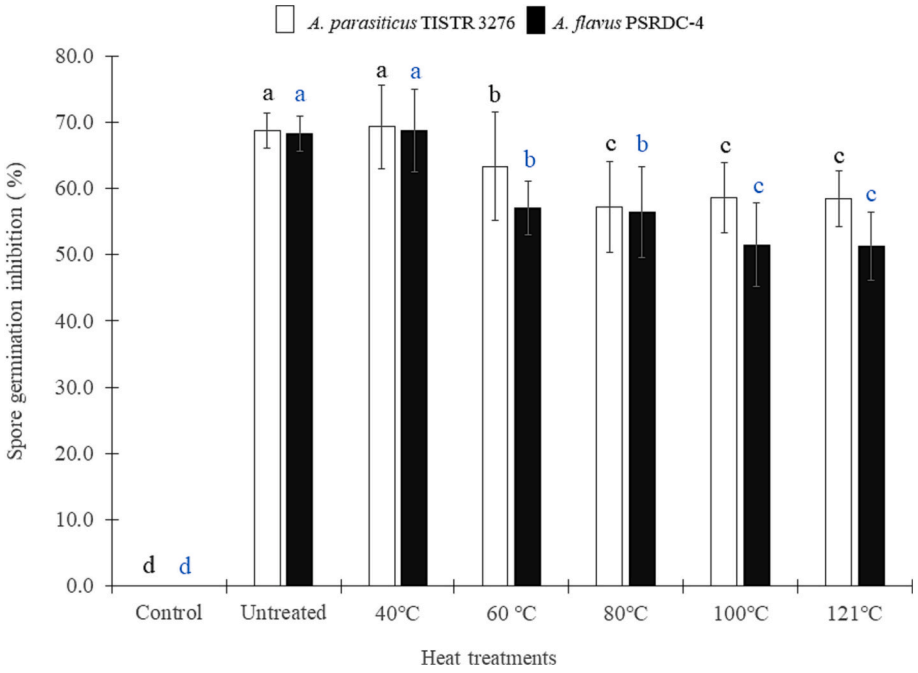


Fig. 1. Effect of different incubation temperatures (ranging from 40 °C to 121 °C) of culture filtrates SKRU-01 on the spore germination inhibition of *A. parasiticus* TISTR 3276 (*white bar*) and *A. flavus* PSRDC-4 (*black bar*) strains after an incubation at 30 °C for 24 h.
Different letters above the bars indicate no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$) between culture filtrates SKRU-01 treated at temperatures ranging from 40 °C to 121 °C against both strains TISTR 3276 and PSRDC-4.

autoclaving. This difference suggests the presence of heat-sensitive antifungal compounds in the culture filtrates SKRU-01, contributing to the observed reduction in inhibition. The existence of heat-sensitive antifungal compounds indicates the potential presence of sensitive bioactive compounds within the culture filtrates SKRU-01, susceptible to damage or denaturation at higher temperatures. This finding implies that *T. asperelloides* SKRU-01 culture filtrates' antifungal properties likely result from a combination of various compounds with varying thermal stability. Moreover, the observed antifungal activity of the culture filtrates SKRU-01 can be attributed, at least in part, to the substantial production of secondary antifungal compounds (Stracquadanio et al., 2020). These secondary compounds, similar to antibiotics commonly found in numerous *Trichoderma* species such as trichogin, trichorzins, harzianins, trichotoxin, trichokindins (Tyskiewicz et al., 2022), gliovirin, gliotoxin, viridin, pyrones, peptaibols (Vey et al., 2001), trichocellins (Tyskiewicz et al., 2022), tanshinone II A, and tanshinone I (Ming et al., 2012) are well-known for their antagonistic effects against fungal pathogens. These pathogens include *Botrytis cinerea* (Zaid et al., 2022), *Pythium ultimum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* (Vargas et al., 2014), *Fusarium moniliforme*, *F. culmorum*, *F. graminearum*, *F. oxysporum*, *Alternaria solani*, and *Penicillium expansum* (Tyskiewicz et al., 2022).

The in vitro inhibition mechanism involves thermally stable secondary antifungal compounds that hinder fungal spore germination and further restrict pathogen proliferation. These findings highlight the potential of culture filtrates SKRU-01 as a promising biocontrol agent against fungal pathogen. Its ability to produce a combination of heat-sensitive and thermally stable antifungal compounds makes it a robust candidate for use in agricultural and environmental applications. In addition, our studies have revealed that the antifungal agents synthesized by SKRU-01 exhibit remarkable heat resistance, withstanding temperatures of up to 121 °C. Consequently, we hypothesize that the primary mechanism responsible for inhibiting aflatoxin-producing fungi arises from the bioactive compounds rather than enzymatic activity. To substantiate this hypothesis, our future research will concentrate on studying the specific bioactive compounds and enzymes influencing fungal inhibition. This comprehensive approach aims to enhance our understanding of fungal inhibitory mechanisms, potentially providing valuable insights for the development of novel and effective biocontrol strategies against fungal pathogens.

In this study, we explored the AFB_1-degrading capabilities of culture filtrates SKRU-01, focusing on both their heat-stable (40–121 °C) and non-heat-treated forms. The investigation revealed that all treatments effectively degraded AFB_1, with the non-heat-treated culture filtrates SKRU-01 (86.41 %) demonstrating higher efficiency compared to the autoclaved culture filtrates (63.91 %) at 96 h incubation. These results indicate that the heat treatment might have partially disintegrated the molecules present in culture filtrates SKRU-01, leading to a reduced AFB_1 degradation efficiency. Comparing the reported AFB_1 degradation abilities of other antagonistic microorganisms, the degradation efficiency of culture filtrates SKRU-01 is similar to that of *S. philanthi* RL-1-178 (>85 %) (Boukaew et al., 2023a), with higher than that of *B. subtilis* UTBSP1 (78.39 %) (Farzaneh et al., 2012), *B. amyloliquefaciens* WF2020 (71.01 %) (Chen et al., 2022), *B. amyloliquefaciens* UTB2 (68.4 %) (Siahmoshteh et al., 2017), and *B. subtilis* UTB3 (70.2 %) (Siahmoshteh et al., 2017), while slightly lower than that of *Nocardia corynebacterioides* DSM 20151 (>90 %) (Teniola et al., 2005). These findings highlight significant variations in AFB_1 degradation efficiency among different strains, indicating that the ability to degrade mycotoxins varies significantly between microbial species. The observed high efficiency of non-heat-treated culture filtrates SKRU-01 suggests its potential as a promising candidate for AFB_1 biodegradation applications. Understanding the differences in degradation efficiency among various microorganisms is crucial for selecting the most suitable strains for practical applications in mycotoxin detoxification and ensuring food and feed safety. Further research and optimization of culture conditions may enhance the AFB_1 degradation potential of culture filtrates SKRU-01, making it a valuable asset in mycotoxin management strategies.

In our study, we aimed to explore a novel approach to tackle aflatoxin contamination in grains, particularly focusing on peanut kernels, by utilizing bioactive metabolites from *T. asperelloides* SKRU-01. Previous research conducted by Reddy et al. (2009) has already reported the

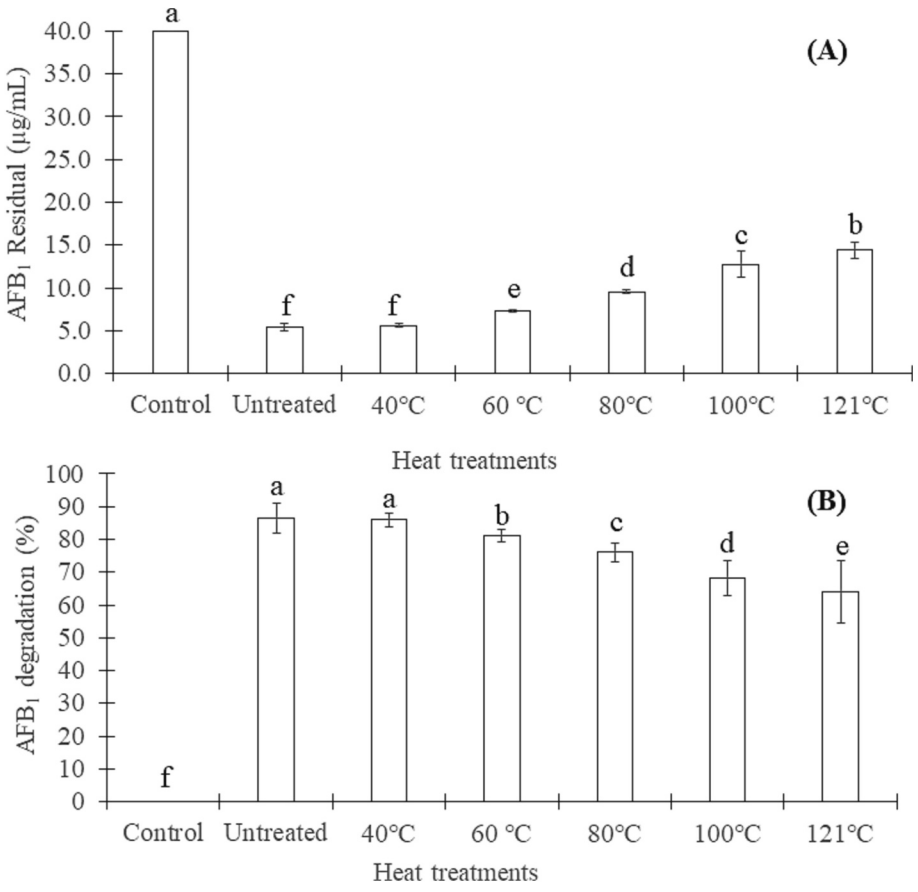


Fig. 2. AFB_1 degradation activity by thermal stability testing (ranging from 40 °C to 121 °C) of the culture filtrates SKRU-01 in PDB medium during 96 h incubation at 30 °C. Panel (A) displays the residual concentration of AFB_1 measured in µg/mL, while panel (B) presents the percentage of AFB_1 degradation. The initial concentration of AFB_1 used in the experiment was 40 µg/mL. Reported values represent means ± standard deviation from three replicates.
Different letters above the bars indicate no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$) between culture filtrates SKRU-01 treated at temperatures ranging from 40 °C to 121 °C in terms of their impact on the residual concentration of AFB_1 and the percentage of AFB_1 degradation.

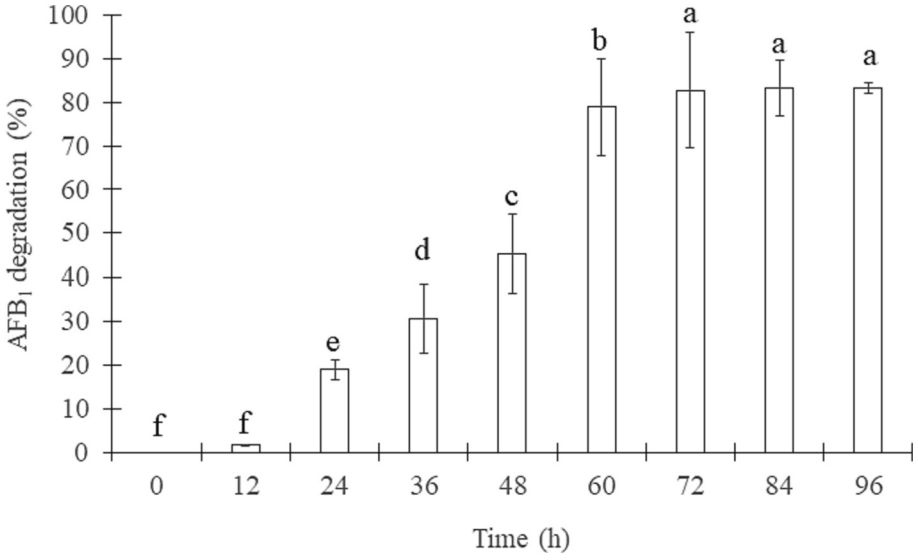


Fig. 3. Time-dependent AFB_1 degradation activity of culture filtrates SKRU-01 *in vitro*. Initial AFB_1 concentration: 40 µg/mL. Reported values represent means ± standard deviation from three replicates.
Different letters above the bars indicate no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$) in the impact of incubation time (0–96 h) on the percentage of AFB_1 degradation.

Table 5
HPLC-FLD method validation parameters for measurement of AFB_1 concentration.

Mycotoxin	Linearity			LOD (μg/kg)	LOQ (μg/kg)
	Range (μg/kg)	Equation	R^2		
AFB_1	5–100	y = 3.56× + 0.09	0.9995	0.9	1.2
Mycotoxin (spiking level, (μg/kg))	Recovery (%)	RSD_r % intra-day	RSD_R % inter-day		
20	106.73	3.24	1.68		
40	103.27	1.02	3.47		
100	92.89	5.10	6.58		

application of these bioactive metabolites to reduce aflatoxin contamination. However, our research stands apart from these previous reports as we specifically investigated the efficacy of thermal stability *Trichoderma* antifungal agents in providing fungal protection for peanut kernels. The consideration of thermal stability is of paramount importance, as it enables these antifungal agents to maintain their efficacy under a wide range of temperature conditions. This characteristic is particularly relevant and practical in real-world agricultural scenarios where fluctuating temperatures are common.

In peanut farming and processing, substantial temperature variations often happened. Peanuts experience a wide temperature range during the growing season, with daytime highs exceeding 32 °C (Puppala et al., 2023) and nighttime lows dropping to approximately 12–15 °C (Wang et al., 2021) in many peanut-producing regions. Post-harvest and processing phases can subject peanuts to elevated temperatures, especially

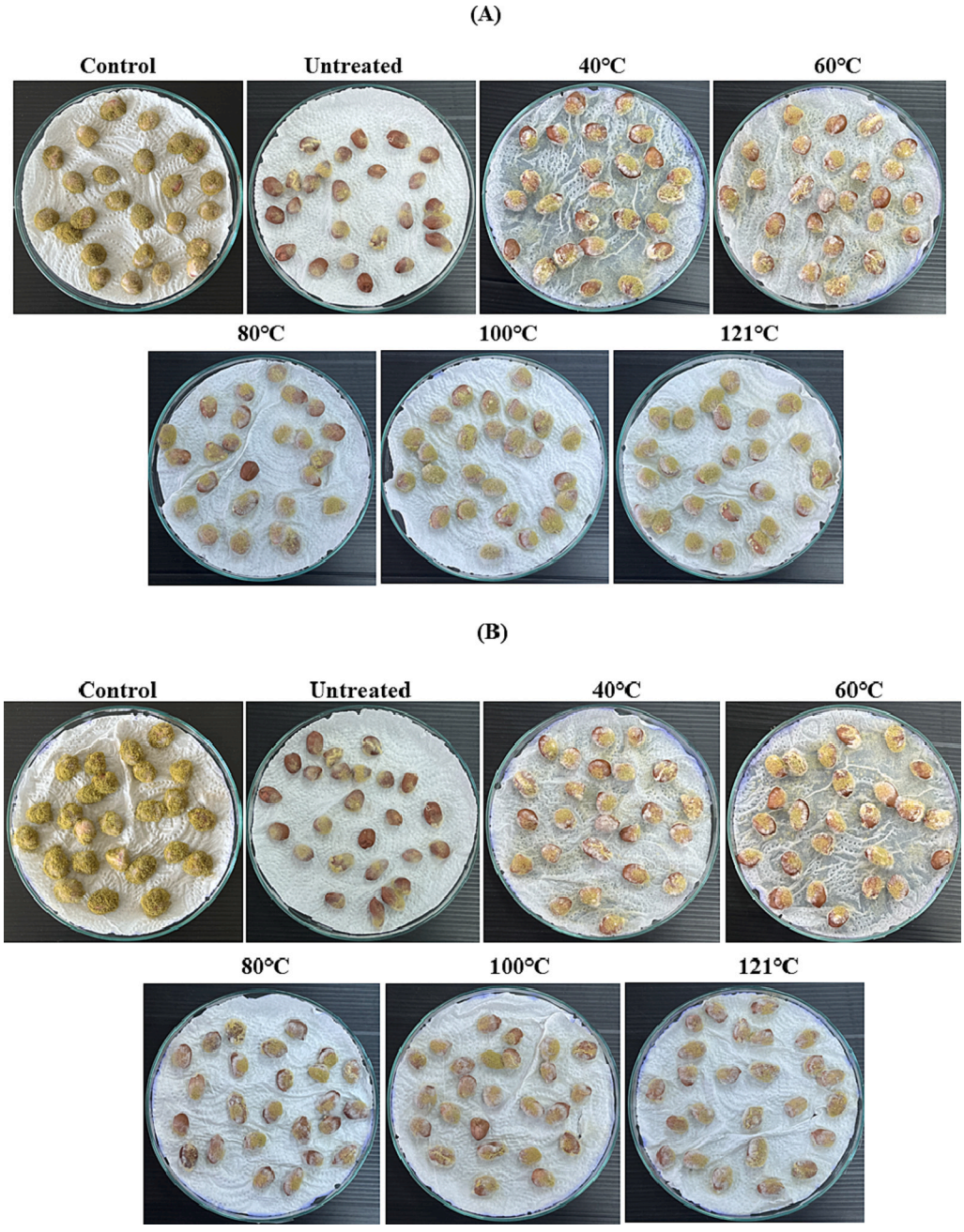


Fig. 4. Growth of (A) *A. parasiticus* TISTR 3276 and (B) *A. flavus* PSRDC-4 in peanuts treated with sterile distilled water (control) and thermal stability testing (ranging from 40 °C to 121 °C) of the culture filtrates SKRU-01 after storage at 30 °C for 5 d.

Table 6
Biocontrol of (A) *A. parasiticus* TISTR 3276 and (B) *A. flavus* PSRDC-4 in peanuts using heat-stable bioactive compounds in culture filtrates SKRU-01 after 5 d incubation at 30 °C.

(A)

Treatments	Viable spores (log spores/g)	Spore reduction (%)	AFB_1 production (μg/kg)
Control	$3.87^{a} \pm 0.68$	–	$148.57^{a} \pm 7.08$
Non-treated	$1.35^{f} \pm 0.51$	65.12	$17.64^{f} \pm 1.54$
40 °C	$1.39^{f} \pm 0.07$	64.08	$19.42^{ef} \pm 3.12$
60 °C	$1.47^{e} \pm 0.12$	59.43	$21.18^{e} \pm 1.23$
80 °C	$1.65^{d} \pm 0.33$	57.36	$29.56^{d} \pm 0.89$
100 °C	$1.72^{c} \pm 0.45$	50.39	$36.75^{c} \pm 2.13$
121 °C	$1.89^{b} \pm 0.29$	45.22	$55.19^{b} \pm 3.17$

(B)

Treatments	Viable spores (log spores/g)	Spore reduction (%)	AFB_1 production (μg/kg)
Control	$4.12^{a} \pm 0.45$	–	$189.45^{a} \pm 2.16$
Non-treated	$1.57^{g} \pm 0.21$	61.89	$19.30^{d} \pm 0.58$
40 °C	$1.61^{f} \pm 0.24$	60.92	$20.01^{d} \pm 1.32$
60 °C	$1.68^{e} \pm 0.11$	59.22	$22.36^{d} \pm 1.41$
80 °C	$2.07^{d} \pm 0.39$	49.76	$48.59^{c} \pm 2.55$
100 °C	$2.23^{c} \pm 0.17$	45.87	$51.58^{c} \pm 5.34$
121 °C	$2.59^{b} \pm 0.67$	39.56	$67.11^{b} \pm 2.19$

Note: Reported values represent means ± standard deviation from three replicates. The control used sterile distilled water. Data followed by the same letter within each column show no statistically significant difference after Tukey's HSD test (ANOVA, $p > 0.05$).

during roasting, where temperatures can exceed 149 °C (Lopez-Garcia and Park, 1998; Wagacha and Muthomi, 2008; Puppala et al., 2023). These fluctuations pose significant challenges for peanut agriculture and processing due to their susceptibility to fungal infections, including *A. flavus* and *A. parasiticus*, which can produce aflatoxins under warm and humid conditions (Kabak et al., 2006; Wagacha and Muthomi, 2008; Khan et al., 2021). Aflatoxins jeopardize peanut product quality, safety, and consumer health. Heat-resistant antifungal metabolites effectively mitigate these challenges, providing vital protection to peanuts throughout their growth cycle and processing. Beyond peanuts, these metabolites hold promise for enhancing crop resilience against temperature-induced fungal infections, addressing food security concerns amid climate change (Puppala et al., 2023).

In this study, we investigated the effect of thermal treatments on culture filtrates SKRU-01 at various temperatures (40–121 °C) and their ability to inhibit mold growth and aflatoxin production. The results indicated that all heat-treated treatments inhibited fungal growth and AFB_1 production in peanuts to some degree. Both non-heated and 40 °C heated samples (<19.42 μg/kg) showed that the AFB_1 levels in peanuts remained below the public health concern threshold of 20 μg/kg (Food and Agriculture Organization (FAO), 2004). It is essential to observe, however, that the efficacy of these heat-treated culture filtrates SKRU-01 was lower than that of the untreated (non-heated) samples. This observation implies that even though heat reduced the effectiveness of inhibiting both strains of fungus, at 121 °C, we found that the culture filtrates SKRU-01 remained heat-resistant and retained their remarkable antifungal properties on peanuts. Under high-temperature conditions, this phenomenon may be attributable to the degradation or modification of certain bioactive metabolites or enzymes present in the culture filtrates. Despite the diminished efficacy after heat treatment, it is notable that the heat-treated culture filtrates SKRU-01 still exhibited substantial antifungal activity, providing some protection against mold growth and aflatoxin contamination. This suggests that the bioactive compounds present in culture filtrates SKRU-01 have inherent resilience, as they retain their ability to inhibit fungal growth even after being exposed to elevated temperatures. It is essential to consider the effect of thermal stability on the efficacy of bioactive compounds, particularly when considering their application in actual agricultural settings. It is essential to establish a balance between optimizing antifungal efficacy and preserving the integrity of bioactive metabolites during the application process, as heat treatment may be required in certain situations. Further research is required to obtain a deeper understanding of the specific changes that occur in the bioactive components of the culture filtrates SKRU-01 following heat treatment. Understanding these changes could provide valuable insights for optimizing treatment protocols and ensuring maximal antifungal activity retention. Our study emphasizes the potential of heat-treating the culture filtrates SKRU-01 to inhibit mold growth and aflatoxin production. Although the efficacy of the treated samples was less than that of the untreated samples, the implications of these findings for the development of biocontrol strategies for aflatoxin management are still significant. As research in this area advances, a comprehensive understanding of the effect of thermal treatment on bioactive metabolites will aid in the development and implementation of effective measures to combat aflatoxin contamination in agricultural practices.

In conclusion, the study demonstrates the remarkable heat stability of culture filtrates SKRU-01, which retained their antifungal activity and effectively degraded aflatoxin B_1, even at high temperatures. The analysis of AFB_1 was carried out using HPLC-FLD method. The obtained analytical results were highly favorable, demonstrating excellent linearity, specificity, accuracy, precision (both repeatability and reproducibility), as well as reliable analytical limits (LOD and LOQ). The heat-resistant properties of these culture filtrates SKRU-01 make them a promising solution for controlling fungal contamination and reducing mycotoxin levels in peanuts. By preventing mold growth and aflatoxin production, the application of culture filtrates SKRU-01 offers significant implications for ensuring food safety and minimizing health risks associated with aflatoxin exposure in peanuts.

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Sawai Boukaew: Funding acquisition, Conceptualization, Data curation, Supervision, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Wanida Petlamul:** Formal analysis, Writing – review & editing. **Sirasit Srinuanpan:** Writing – review & editing. **Karistsapol Nooprom:** Formal analysis, Writing – review & editing. **Zhiwei Zhang:** Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Data availability

No data was used for the research described in the article.

References

Abd-Alla, M.A., 2005. Effect of acetic acid fumigation on common storage fungi of some medicinal and aromatic seeds. Egypt J. Pathol. 33, 77–86.
Amador, M.L., Jimeno, J., Paz-Ares, L., Cortes-Funes, H., Hidalgo, M., 2003. Progress in the development and acquisition of anticancer agents from marine sources. Ann. Oncol. 14, 1607–1615.

Ayyandurai, M., Akila, R., Manonmani, K., Harish, S., Mini, M.L., Vellaikumar, S., 2023. Deciphering the mechanism of *Trichoderma* spp. consortia possessing volatile organic compounds and antifungal metabolites in the suppression of *Sclerotium rolfsii* in groundnut. Physiol Mol. Plant Pathol. 125, 102005.

Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L., Senghor, A.L., Adhikari, B.N., Cotty, P.J., 2016. Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. World Mycotoxin J. 9, 771–789.

Behiry, S., Soliman, S.A., Massoud, M.A., Abdelbary, M., Kordy, A.M., Abdelkhalek, A., Heflish, A., 2023. *Trichoderma pubescens* elicit induced systemic resistance in tomato challenged by *Rhizoctonia solani*. J. Fungi 9, 167.

Boukaew, S., Petlamul, W., Prasertsan, P., 2020a. Efficacy of *Streptomyces philanthi* RL-1-178 culture filtrate against growth and aflatoxin B_1 production by two aflatoxigenic fungi on maize seeds. Eur. J. Plant Pathol. 156, 1041–1051.

Boukaew, S., Petlamul, W., Prasertsan, P., 2020b. Tuna condensate waste with molasses as a renewable substrate for antifungal compounds by *Streptomyces philanthi* RL-1-178 against aflatoxingenic B_1 (AFB_1) *Aspergillus flavus*. Waste Biomass Valoriz. 11, 1321–1331.

Boukaew, S., Prasertsan, P., Mahasawat, P., Sriyatep, T., Petlamul, W., 2023a. Efficacy of the antifungal metabolites of *Streptomyces philanthi* RL-1-178 on aflatoxin degradation with its application to prevent aflatoxigenic fungi in stored maize grains and identification of the bioactive compound. World J. Microbiol. Biotechnol. 39, 24.

Boukaew, S., Mahasawat, P., Petlamul, W., Chuprom, J., Sattayasamitsathit, S., Surinkaew, S., Chuprom, J., Prasertsan, P., 2023b. Application of antifungal metabolites from *Streptomyces philanthi* RL-1-178 for maize grain coating formulations and their efficacy as biofungicide during storage. World J. Microbiol. Biotechnol. 39, 157.

Boukaew, S., Zhang, Z., Prasertsan, P., Igarashi, Y., 2023c. Antifungal and antiaflatoxigenic mechanism of freeze-dried culture filtrate of *Streptomyces philanthi* RL-1-178 on the two aflatoxigenic fungi and identification of its active component. J. Appl. Microbiol. 134, 1–9.

Boukaew, S., Kumla, J., Prasertsan, P., Cheirsilp, B., Petlamul, W., Srinuanpan, S., 2023d. *In vitro* and in situ antifungal properties of a *Trichoderma asperelloides* SKRU-01 against aflatoxigenic aspergillus species. Food Control 154, 110025.

Branà, M.T., Cimmarusti, M.T., Haidukowski, M., Logrieco, A.F., Altomare, C., 2017. Bioremediation of aflatoxin B_1-contaminated maize by king oyster mushroom (*Pleurotus eryngii*). PLoS One 12, e0182574.

Calistru, C., McLean, M., Berjak, P., 1997. *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species—a study of the production of extracellular metabolites by *Trichoderma* species. Mycopathologia 137, 115–124.

Campos-Avelar, I., Colas de la Noue, A., Durand, N., Cazals, G., Martinez, V., Strub, C., Fontana, A., Schorr-Galindo, S., 2021. *Aspergillus flavus* growth inhibition and aflatoxin B_1 decontamination by *Streptomyces* isolates and their tetabolites. Toxins 13, 340.

Chen, G., Fang, Q., Liao, Z., Xu, C., Liang, Z., Liu, T., Zhong, Q., Wang, L., Fang, X., Wang, J., 2022. Detoxification of aflatoxin B_1 by a potential probiotic *Bacillus amyloliquefaciens* WF2020. Front. Microbiol. 13, 891091.

Chet, I., Baker, R., 1981. Isolation and biological potential of *Trichoderma hamatum* from soil naturally suppressive of *Rhizoctonia solani*. Phytopathol 71, 268–290.

Chiuraise, N., Yobo, K.S., Laing, M.D., 2015. Seed treatment with Trichoderma harzianum strain kd formulation reduced aflatoxin contamination in groundnuts. J. Plant Dis. Prot. 122, 74–80.

Choochuay, S., Phakam, J., Jala, P., Maneeboon, T., Tansakul, N., 2018. Determination of aflatoxin B_1 in feedstuffs without clean-up step by high-performance liquid chromatography. Int. J. Anal. Chem. 1–8.

Dini, I., Alborino, V., Lanzuise, S., Lombardi, N., Marra, R., Balestrieri, A., Ritieni, A., Woo, S.L., Vinale, F., 2022. *Trichoderma* enzymes for degradation of aflatoxin B_1 and ochratoxin A. Molecules 27, 3959.

Farzaneh, M., Shi, Z.-Q., Ghassempour, A., Sedaghat, N., Ahmadzadeh, M., Mirabolfathy, M., Javan-Nikkhah, M., 2012. Aflatoxin B_1 degradation by *Bacillus subtilis* UTBSP1 isolated from pistachio nuts of Iran. Food Control 23, 100–106.

Food and Agriculture Organization (FAO), 2004. Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food Nutr. Pap. 81.

Gomaa, E.Z., Housseiny, M.M., Omran, A.A.A.-K., 2019. Fungicidal efficiency of silver and copper nanoparticles produced by *Pseudomonas fluorescens* ATCC 17397 against four *Aspergillus* species: a molecular study. J. Clust. Sci. 30, 181–196.

Gong, A.-D., Lei, Y.-Y., He, W.-J., Liao, Y.-C., Ma, L., Zhang, T.-T., Zhang, J.-B., 2022. The inhibitory effect of *Pseudomonas stutzeri* YM6 on *Aspergillus flavus* growth and aflatoxins production by the production of volatile dimethyl trisulfide. Toxins 14, 788.

Gong, A.-D., Song, M.-G., Wang, H.-L., Wang, G.-Z., Wang, J.-H., Zhang, J.-B., 2023. Inhibitory effect of volatile organic compounds from *Bacillus fexus* TR-1 against *Aspergillus favus* and aflatoxins in grains during storage. BioControl 68, 181–190.

Hackbart, H.C.S., Machado, A.R., Christ-Ribeiro, A., Prietto, L., Badiale-Furlong, E., 2014. Reduction of aflatoxins by *Rhizopus oryzae* and *Trichoderma reesei*. Mycotoxin Res. 30, 141–149.

Hermosa, R., Cardoza, R.E., Rubio, M.B., Gutiérrez, S., Monte, E., 2014. Secondary metabolism and antimicrobial metabolites of *Trichoderma*. Biotechnol. Biol. Trichoderma 125–137.

Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis. 87, 4–10.

Kabak, B., Dobson, A.D.W., Var, I., 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. Crit. Rev. Food Sci. Nutr. 46, 593–619.

Khan, R., Ghazali, F.M., Mahyudin, N.A., Samsudin, N.I.P., 2021. Biocontrol of aflatoxins using non-aflatoxigenic *Aspergillus flavus*: a literature review. J. Fungi 7, 381.

Li, W.R., Shi, Q.S., Ouyang, Y.S., Chen, Y.B., Duan, S.S., 2013. Antifungal effects of citronella oil against *Aspergillus niger* ATCC 16404. Appl. Microbiol. Biotechnol. 97, 7483–7492.

Li, S., Xu, X., Zhao, T., Ma, J., Zhao, L., Song, Q., Sun, W., 2022. Screening of *Bacillus velezensis* E2 and the inhibitory effect of its antifungal substances on *Aspergillus flavus*. Foods 11, 140.

Lopez-Garcia, R., Park, D., 1998. Effectiveness of post-harvest procedures in management of mycotoxin hazards. In: Bhatnagar, D., Sinha, S. (Eds.), Mycotoxins in Agriculture and Food Safety. Marcel Dekker, New York, USA, pp. 407–433.

Ma, F., Cai, X.F., Mao, J., Yu, L., Li, P.W., 2021. Adsorptive removal of aflatoxin B_1 from vegetable oils via novel adsorbents derived from a metal-organic framework. J. Hazard. Mater. 412, 125170.

Madbouly, A.K., Rashad, Y.M., Ibrahim, M.I.M., Elazab, N.T., 2023. Biodegradation of Aflatoxin B_1 in maize grains and suppression of its biosynthesis-related genes using endophytic *Trichoderma harzianum* AYM3. J. Fungi 9, 209.

Mason, P.G., Hill, M., Smith, D., Silvestri, L.C., Weyl, P., Brodeur, J., Vitorino, M.D., 2023. Best practices in the use and exchange of microorganism biological control genetic resources. BioControl 68, 311–327.

Ming, Q., Han, T., Li, W., Zhang, Q., Zhang, H., Zheng, C., 2012. Tanshinone II A and tanshinone I production by *Trichoderma atroviride* D16, an endophytic fungus in *Salvia miltiorrhiza*. Phytomedicine 19, 330–333.

Palonen, H., Tjerneld, F., Zacchi, G., Tenkanen, M., 2004. Adsorption of *Trichoderma reesei* CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin. J. Biotechnol. 107, 65–72.

Pribowo, A., Arantes, V., Saddler, J.N., 2012. The adsorption and enzyme activity profiles of specific *Trichoderma reesei* cellulase/xylanase components when hydrolyzing steam pretreated corn Stover. Enzym. Microb. Technol. 50, 195–203.

Puppala, N., Nayak, S.N., Sanz-Saez, A., Chen, C., Devi, M.J., Nivedita, N., Bao, Y., He, G., Traore, S.M., Wright, D.A., Pandey, M.K., Sharma, V., 2023. Sustaining yield and nutritional quality of peanuts in harsh environments: physiological and molecular basis of drought and heat stress tolerance. Front. Genet. 14, 1121462.

Reddy, K.R.N., Reddy, C.S., Muralidharan, K., 2009. Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control 20, 173–178.

Ren, X., Branà, M.T., Haidukowski, M., Gallo, A., Zhang, Q., Logrieco, A.F., Li, P., Zhao, S., Altomare, C., 2022. Potential of *Trichoderma* spp. for biocontrol of aflatoxin-producing *Aspergillus flavus*. Toxins 14, 86.

Siahmoshteh, F., Siciliano, I., Banani, H., Hamidi-Esfahani, Z., Razzaghi-Abyaneh, M., Gullino, M.L., Spadaro, D., 2017. Efficacy of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in the control of *Aspergillus parasiticus* growth and aflatoxins production on pistachio. Int. J. Food Microbiol. 254, 47–53.

Siciliano, I., Spadaro, D., Prelle, A., Vallauri, D., Cavallero, M., Garibaldi, A., Gullino, M., 2016. Use of cold atmospheric plasma to detoxify hazelnuts from aflatoxins. Toxins (Basel) 8, 125.

Spadaro, D., Droby, S., 2016. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. Trends Food Sci. Technol. 47, 39–49.

Spadaro, D., Garibaldi, A., 2017. Containment of mycotoxins in the food chain by using decontamination and detoxification techniques. In: Gullino, M.L., Stack, J., Fletcher, J., Mumford, J. (Eds.), Practical Tools for Plant and Food Biosecurity. Springer, Dordrecht, The Netherlands, pp. 163–177.

Stracquadanio, C., Quile,s, J.M., Meca, G., Cacciola, S.O., 2020. Antifungal activity of bioactive metabolites produced by *Trichodermaa sperellum* and *Trichoderma atroviride* in liquid medium. J. Fungi (Basel) 6, 263.

Teniola, O.D., Addo, P.A., Brost, I.M., Farber, P., Jany, K.D., Alberts, J.F., van Zyl, W.H., Steyn, P.S., Holzapfel, W.H., 2005. Degradation of aflatoxin B_1 by cell-free extracts of *Rhodococcus erythropolis* and *Mycobacterium fluoranthenivorans* sp. nov. DSM 44556(T). Int. J. Food Microbiol. 105, 111–117.

Tosch, D., Waltking, A.E., Schlesier, J.F., 1984. Comparison of liquid chromatography and high performance thin layer chromatography for determination of aflatoxin in peanut products. J. Assoc. Anal. Chem. 67, 337–339.

Tyskiewicz, R., Nowak, A., Ozimek, E., Jaroszuk-Scisel, J., 2022. *Trichoderma*: the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int. J. Mol. Sci. 23, 2329.

Vargas, W.A., Mukherjee, P.K., Laughlin, D., Wiest, A., Moran-Diez, M.E., Kenerley, C.M., 2014. Role of gliotoxin in the symbiotic and pathogenic interactions of *Trichoderma virens*. Microbiol 160, 2319–2330.

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., Valéro, J.R., 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. Biochem. Eng. J. 37, 1–20.

Vey, A., Hoagland, R.E., Butt, T.M., 2001. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Toxic Metabolites of Fungal Biocontrol Agents. Fungi as Biocontrol Agents: Progress, Problems and Potential. CAB International, Bristol, pp. 311–346.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L., Lorito, M., 2008. Trichoderma–plant–pathogen interactions. Soil Biol. Biochem. 40, 1–10.

Wagacha, J.M., Muthomi, J.W., 2008. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol. 124, 1–12.

Wang, C., Wang, Z., Wei, Y., Tang, Y., Wang, F., Han, H., Sun, T., 2021. Effect of variety and seed dressing on emergence of high-oleic peanut under low temperature and high soil humidity conditions. Oil Crop Sci. 6, 164–168.

Whipps, J.M., Lumsden, R.D., 1991. Biological control of *Pythium* species. Biocontrol Sci. Tech. 1, 75–90.

World Health Organization, 1993. International Agency for Research on Cancer. Aflatoxins. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; IARC Press: Lyon, France, 56, pp. 245–395.

Xia, X., Zhang, Y., Li, M., Garba, B., Zhang, Q., Wang, Y., Zhang, H., Li, P., 2017. Isolation and characterization of a *Bacillus subtilis* strain with aflatoxin B_1 biodegradation capability. Food Control 75, 92–98.

Xie, H., Wang, X., Zhang, L., Wang, T., Zhang, W., Jiang, J., Chang, P.-K., Chen, Z.-Y., Bhatnager, D., Zhang, Q., Li, P., 2018. Monitoring metabolite production of aflatoxin biosynthesis by Orbitrap Fusion mass spectrometry and D-optimal mixture design method. Anal. Chem. 90, 14331–14338.

Yang, E.J., Chang, H.C., 2010. Purification of a new antifungal compound produced by *Lactobacillus plantarum* AF_1 isolated from kimchi. Int. J. Food Microbiol. 139, 56–63.

Yuan, S., Wu, Y., Jin, J., Tong, S., Zhang, L., Cai, Y., 2023. Biocontrol capabilities of *Bacillus subtilis* E11 against *Aspergillus flavusin vitro* and for dried red chili (*Capsicum annuum* L.). Toxins 15, 308.

Yue, X., Ren, X., Fu, J., Wei, N., Altomare, C., Haidukowski, M., Logrieco, A.F., Zhang, Q., Li, P., 2022. Characterization and mechanism of aflatoxin degradation by a novel strain of *Trichoderma reesei* CGMCC3.5218. Front. Microbiol. 13, 1003039.

Zafar, H., Shaukat, S.S., Rao, T.A., 2013. Antagonistic activity of cultural filtrates of five *Trichoderma* species against pathogenic fungus *Alternaria solani*. Int. J. Biol. Biotechnol. 10, 547–551.

Zafra, G., Cortés-Espinosa, D.V., 2015. Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species: a mini review. Environ. Sci. Pollut. Res. Int. 22, 19426–19433.

Zaid, R., Koren, R., Kligun, E., Gupta, R., Leibman-Markus, M., Mukherjee, P.K., Kenerley, C.M., Bar, M., Horwitz, B.A., 2022. Gliotoxin, an immunosuppressive fungal metabolite, primes plant immunity: evidence from *Trichoderma virens*-tomato interaction. ASM 13, 1–19.