

Assessment of Aflatoxin B1 in Common Spices and Evaluation of Sustainable Detoxification Methods

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ABSTRACT

It assesses aflatoxin levels in various spices used in food preparation. It evaluates the effectiveness of decontamination methods, and 5 samples for each spice, such as *Cuminum cyminum* (cumin), *Foeniculum vulgare* (fennel), *Capsicum annuum* (red pepper), *Coriandrum sativum* (coriander), *Syzygium aromaticum* (clove), *Trachyspermum ammi* (carom), *Cinnamomum verum* (cinnamom), and *Curcuma longa* (turmeric), were collected from different markets. Aflatoxins were quantified by thin-layer chromatography (TLC), and *F. vulgare* contained 99.72 ppb aflatoxin B1, exceeding the safe limit of 20 ppb. Other spices do not detect aflatoxins, and methods of decontamination: physical (cold water, boiled water), chemical (citric acid, ammonium hydroxide), and biological (Moringa oil, Black Seed oil) were evaluated on the extent of aflatoxin reduction. Citric acid (C₆H₈O₇) and ammonium hydroxide (NH₄OH) reduced aflatoxins by 52%-64% and 44.9%-67%, followed by Black Seed oil, which was the most effective at 15% with a 72.45% reduction. Moisture content data were analysed using One-Way ANOVA, with an F-value of 4.26 and a p-value of 0.03, indicating significant differences in moisture content across spices. In Tukey's HSD test, it was also found that *F. vulgare* and *C. annuum* have significantly (p=0.01) higher moisture content than *C. cyminum* and *C. longa*. Two-way ANOVA showed a significant effect of spice type and measurement time on the moisture content of palm oil F = 5.34, p = 0.02). Sensory and nutritional analyses indicated that decontamination had minimal effect on sensory characteristics and nutrient composition of products, and among biological interventions, Black Seed oil was found to be most effective for maintaining product quality in terms of sensory characteristics and nutrient value. The research highlights the risk of aflatoxin contamination in *F. vulgare* and demonstrates the effectiveness of biological interventions, especially black seed oil, in detoxifying spices while retaining their quality, offering viable strategies to ensure the safety of spice consumption.

Keywords: Detoxification strategies; Mycotoxin contamination; Moisture content analysis; Thin Layer Chromatography; Sustainable food safety.

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INTRODUCTION

Aflatoxins are toxic secondary metabolites that are commonly produced by *Aspergillus flavus* and *Aspergillus parasiticus* under appropriate conditions that favor growth, such as warm and humid temperatures (Amaike et al., 2011; Ramadan et al., 2022).

and are very resistant to standard processing techniques, remaining even after drying, heating, and cooking (Koszucka et al., 2019). They also possess considerable hepatotoxic and carcinogenic properties and are a major global food safety concern (Ashraf et al., 2023). It has also caused both acute and chronic diseases such as liver damage, immunosuppression, and affects the development of the child (Bbosa et al., 2013; Cunningham et al., 2005). Group 1 carcinogen according to IARC, aflatoxin B1 (AFB1) is known to be the primary cause of hepatocellular carcinoma with respect to high intake and hepatitis B virus infection (Panel et al., 2020). Aflatoxins cause a considerable burden of liver cancer worldwide, particularly in low and middle-income countries, because of inefficient food safety systems (El Saghir et al., 2014), and AFB1 is considered the most potent, causing death in large doses and severe liver injury (Singh et al., 2021). Despite contamination, it is difficult to detect in developing regions due to poor laboratory infrastructure and regulatory framework (Meneely et al., 2023). Most of the common detection methods, like HPLC, ELISA, and TLC, were not feasible in low-resource regions in terms of testing local and non-branded food products, which leads to their undetected access to the public (Iqbal et al., 2014; Shephard et al., 2014; Makinde et al., 2020; Hlaing, 2023) and thus contaminates the consumers without detection (Oktapodas Feiler et al., 2022). Even though many spices are at high risk of fungal colonization as well as the presence of fungal toxins (Kumar et al., 2021), most of the attention has been paid to the aflatoxin contamination in staple foods only, and spices have been ignored almost entirely despite having a similar risk of fungal invasion and mycotoxin production (Glodjinon et al., 2019). Many spices, including *C. annuum*, pepper, and *F. vulgare*, are used as staple foods and are stored for long periods under inappropriate environmental conditions, thus increasing the risk of contamination (Glodjinon et al., 2019). Non-branded spices are easily affected by inadequate quality controls and regulations, leading to significant risks (Akhtar et al., 2020). Different types of detoxification methods, such as physical, chemical, and irradiation methods, have been studied but are limited by their cost, adverse effects on food quality and taste, and environmental concerns (Mostashari et al., 2022). Even though the effectiveness of some chemical detoxification methods is observed, toxicity and residue-related issues have caused controversy

about using them for detoxification (Purohit et al., 2023; Wang et al., 2019). So, biological detoxification using microorganisms is the most promising approach and would not affect food quality as compared to chemical detoxification (Hathout and Aly, 2014). Different genera and species of bacteria, especially those in *Bacillus* and *Lactobacillus*, can degrade AFB1 and reduce its toxicity (Huang et al., 2018). Therefore, in this study, the effect of biological detoxification on spices, particularly non-branded ones commonly used by people with limited resources, will be examined to identify an effective, cost-effective approach that does not compromise food safety or the quality of spices, while ensuring the safe use of microorganisms.

MATERIALS AND METHODS

The research assessed the efficacy of physical, chemical, and biological methods for detoxifying aflatoxin in unbranded spices purchased from the local market in Lahore, Pakistan. The methodology included sample collection, aflatoxin detection, detoxification processes, analysis of all data to reduce contamination levels, and evaluation of food safety aspects and the sensory properties of the treated samples. All analyses were performed at the Food and Biotechnology Research Center (FBRC) under Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore, and the Biotechnology Research Laboratory of Lahore College for Women University (LCWU) using sophisticated instruments such as High-Performance Liquid Chromatography (HPLC) systems and Thin Layer Chromatography (TLC) set-ups.

Sample Collection and Preparation

Non-branded samples of spices, including the spices *C. cyminum*, *F. vulgare*, *C. annuum*, *C. sativum*, *S. aromaticum*, *T. ammi*, *C. verum*, and *C. longa*, were collected from seven key markets in Lahore, i.e., Lohari Market, Shalmi Market, Akbari Mandi, Ichhra Bazaar, Shah Alam Market, Anarkali Bazaar, and R.A Bazar, as shown in Figure 1. A quantity of 500g of each spice was collected from several suppliers and organizations to obtain a single composite sample, thereby minimizing variability and ensuring representative results. The spices were ground to a fine powder using a sterile grinder and then stored in an airtight, dark container at ambient temperature (22-25°C) to prevent photodegradation of any possible aflatoxin contaminants (Abuagela, 2017).



Figure 1. Samples of spices were collected from different markets of Lahore for the assessment of the proliferation of fungi due to environmental conditions and other factors. The samples included (a) *C. sativum*, (b) *F. vulgare*, (c) *C. verum*, (d) *C. cyminum*, (e) *C. longa*, (f) *S. aromaticum*, (g) *C. annum*, and (h) *T. ammi*.

Aflatoxin Detection and Quantification

The analysis of aflatoxins followed a standard procedure that included extraction, preliminary thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) for quantification. Extraction was done by adding 50 g of each spice sample, 25 g diatomaceous earth, and 25 mL distilled water in a 500 mL conical flask. Chloroform (150 mL) was used as an extraction solvent because it is good for aflatoxin extraction (Iqbal et al., 2017). The mixture was agitated at 200 rpm for 30 min to maximize aflatoxin extraction, then filtered through Whatman No. 1 filter paper. The chloroform extract was then evaporated on a hot plate at 60-70°C until dryness. To confirm the presence of aflatoxin, 0.5 mL of each extract was spotted on a TLC plate coated with silica gel (10 x 5 cm) and run in an anhydrous ether solvent system. TLC plates were observed under UV light (365 nm), where aflatoxins displayed distinct fluorescent spots, indicating their presence and type (Suleiman, 2023).

Quantitative analysis

Quantitative analysis was performed by HPLC, following affinity purification with immunoaffinity columns to reduce co-extracted interferences. Analyte separation was achieved on a reverse-phase C18

column with fluorescence detection at 360 nm (excitation) and 440 nm (emission). Aflatoxin concentrations were calculated by comparing sample peak areas with those of standard aflatoxin solutions, with results expressed in ng/g of spice (Zhang and Banerjee et al., 2020).

Detoxification Techniques and Analysis

Physical Detoxification

In the physical detoxification process, both hot and cold-water treatments were applied to explore the effect of temperature on aflatoxin stability (Mostashari et al., 2022). For the hot-water treatment, 5 g of each contaminated sample was immersed in 100 mL of boiling water (100°C) and shaken for 30 min. After treatment, the samples were filtered, extracted with chloroform, and subjected to TLC to determine residual aflatoxin levels. Cold-water treatment followed the same protocol at 4°C to investigate the stability of aflatoxins under low-temperature conditions (Chaudhary et al., 2021).

Chemical Detoxification

Chemical detoxification is performed using citric acid ($C_6H_8O_7$) and ammonium hydroxide (NH_4OH), both of which are known for their ability to degrade mycotoxins (Karlovsy et al. 2016). For the treatment with citric acid, 10 g of spice was mixed with 100 mL

of 10 % citric acid solution, agitated for 30 minutes, and then filtered. Chloroform (CHCl₃) extraction and thin-layer chromatography (TLC) were used for the determination of the residual aflatoxins. In the treatment with ammonium hydroxide, 10 g of the spice was treated with 100 mL of 10 % ammonium hydroxide solution, shaken, filtered, and analyzed as described. The objective of both procedures was to disrupt aflatoxin structure by altering pH (Asghar et al., 2016).

Moisture Content Analysis

The moisture content was measured using the standard moisture analyzer to determine the effect of water activity on the stability of aflatoxin and the proliferation of microorganisms in spices. For each sample, 1g was heated to 110°C until a stable mass was reached. The moisture content was determined through the mass loss after drying. This data proved indispensable for elucidating the moisture threshold conducive to aflatoxin proliferation on untreated specimens (Ali et al., 2016).

Sensory and Nutritional Quality Assessment Post-Detoxification

Following the detoxification procedure, sensory and nutritional studies were conducted to assess the effects on consumer acceptability and the nutritional quality of the spices. The sensory evaluation used a five-point hedonic scale, and trained panelists also evaluated each sample for color, aroma, and flavor retention. Furthermore, the nutritional composition of the detoxified product was quantified by measuring protein and fat content using the standard Kjeldahl extraction method and the Soxhlet extraction method, respectively, to verify that its nutritional product attributes were not affected by the detoxification process. The results of this assessment indicated that the detoxified spices met the quality standards required for consumer acceptance (Chaudhary et al., 2021; Salem et al., 2018).

Statistical Analysis

The effectiveness of the detoxification methods was shown in statistical analyses. Aflatoxin concentrations in treated and untreated samples were analyzed using paired t-tests, and comparisons among several groups were performed using analysis of variance (ANOVA). The calculated reduction percentages were used to quantify detoxification efficiency over time. Statistical significance was assessed at $p \leq 0.05$, and confidence intervals were

presented for each result to validate the reliability of the findings (Hussain et al., 2020). Data normalization techniques were used to address variance in baseline contamination levels across spice types, thereby confirming the robustness of each detoxification method.

RESULTS

Sample Collection and Preparation

In our results, we identified that 8 of the selected spice samples, comprising *C. cyminum*, *F. vulgare*, *C. annum*, *C. sativum*, *S. aromaticum*, *T. ammi*, *C. verum*, and *C. longa*, had differing moisture content and contamination propensity when collected from different markets of Lahore. We procured samples from Lohari Market, Shalmi Market, Akbari Mandi, Ichhra Bazaar, Shah Alam Market, Anarkali Bazaar, and R.A Bazar, Lahore-Pakistan (Figure 2). Each spice type was purchased from different stores and vendors to capture a comprehensive average portion size in the local market; 500 g of each was purchased. Where necessary, they were each crushed and ground in a sterile grinder to ensure a consistent particle size. To minimize photodegradation and other environmental factors that affect moisture and contaminants in these powdered samples, we stored them in dark, airtight containers at ambient temperatures of 22-25°C. This storage condition enabled the researchers to maintain the purity of potentially sacrosanct compounds, e.g., aflatoxin and to provide a basis for subsequent analyses.

Aflatoxin Detection and Quantification

In the current study, the level of contamination in non-branded spice samples was tested using Thin Layer Chromatography (TLC), a relatively sensitive technique for aflatoxin detection. Specifically, this method enabled the identification of four major types of aflatoxins: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) when exposed to ultraviolet light sources. TLC was used to identify these aflatoxins in spice samples used in cooking practices and to determine their concentrations. The objectives of this work were to evaluate the concentrations of these carcinogenic toxins and to obtain quantitative data on contamination levels in various types of spices. The presented UV-based TLC proved sensitive enough to identify aflatoxins and differentiate among the various aflatoxin types detected in the spice samples.

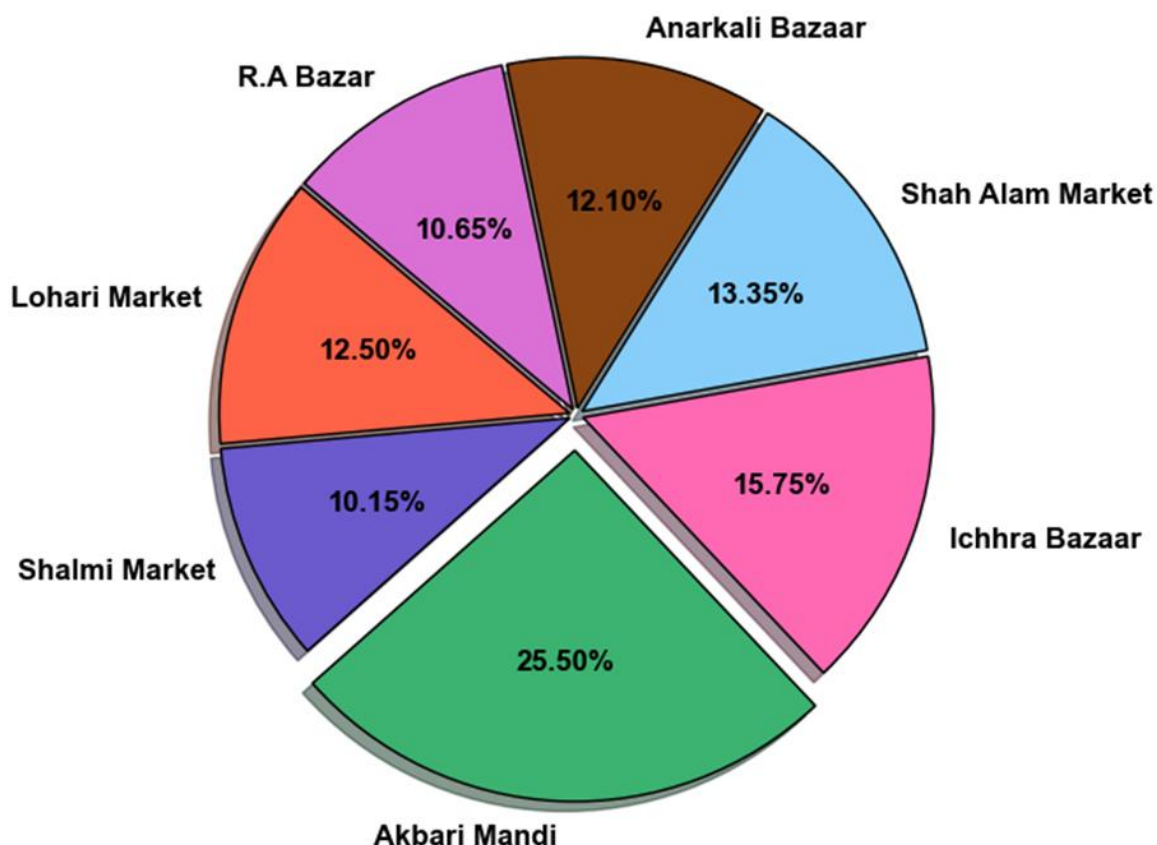


Figure 2. The pie chart shows the distribution of spice samples that were obtained from seven markets. The representation includes the following places - Lohari Market (12.5%), Shalmi Market (10.15%), Akbari Mandi (25.5%, which is the largest segment), Ichhra Bazaar (15.75%), Shah Alam Market (13.35%), Anarkali Bazaar (12.1%), and R.A Bazar (10.65%).

Aflatoxin Estimation in Non-Branded Spice Samples

From the eight non-branded spice samples collected from seven different places A, B, C, D, E, F, G, and H aflatoxin was found positive in only one sample, which means 12.5% in total. The remaining 87.5% of samples (A, C, D, E, F, G, and H) were found to be free of aflatoxin, and thus the investigated spices did not exceed the risk level. Among all the spice samples analyzed for aflatoxin levels, the highest contamination was observed in Sample B (*F. vulgare*), with a level of 99.72 ppb. It also confirms the high levels of AFB1 above which food is not safe for human consumption, according to the set food safety standards. Sample B was subjected to Thin Layer Chromatography (TLC), and the presence of AFB1 in sample B was ascertained by comparing the chromatographic prints of Sample B with those of standard AFLA compounds (AFB1, AFB2, AFG1, and AFG2) as shown in Figure 3. AFB1 showed quite different fluorescence from the other compounds

under ultraviolet light, allowing easy separation of the two compounds and precise ratio calculation. Consequently, aflatoxin B was confirmed in *F. vulgare*, and the sample is dangerous for direct human consumption without prior treatment. Furthermore, the analysis showed that AFB1 is the most potent and most carcinogenic of the aflatoxins, making it necessary to screen foods for its presence. The presence of AFB1 in *F. vulgaris* (Sample B) underscores the need to strengthen quality control measures for spices, as even trace amounts may pose a threat to human health. Although only one of the samples tested was found to be contaminated with aflatoxins, the study helps expand our understanding of contamination in popular spices; however, *F. vulgare* is considered a principal source of aflatoxin contamination. The breakdown of the concentration and all other raw data that have been derived from this analysis are provided in the appendix for ease of reference and reproducibility.

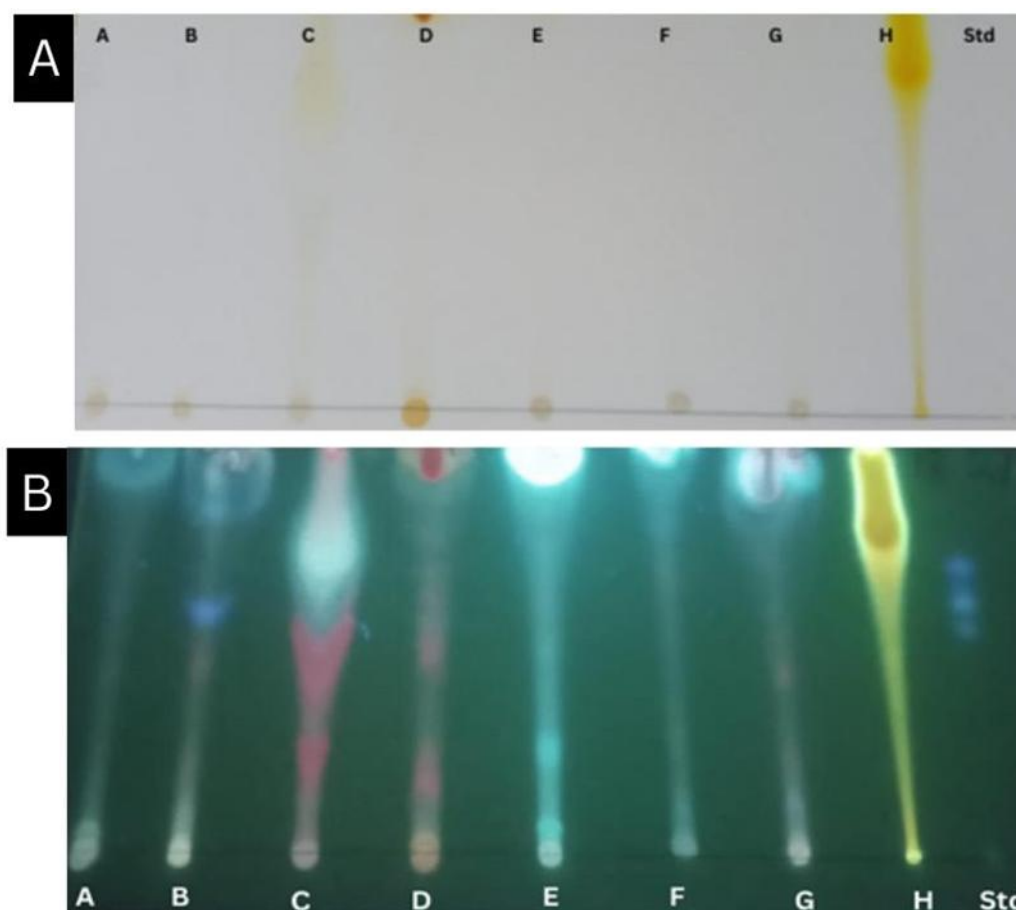


Figure 3. A TLC plate under ultraviolet light shows the high prevalence of aflatoxin B1 in sample B (*F. vulgare*) out of all samples labelled A, B, C, D, E, F, G, and H (eight samples).

Comparative Analysis of Aflatoxin Concentration Using Different Volumes of *F. vulgare* extract

In addition to qualitative identification of aflatoxin occurrence in spices, the experimental results were also quantified to assess the sample volume required for aflatoxin detection. This experiment aimed to compare the volume of *F. vulgare* extract applied (5 μ L, 10 μ L, 15 μ L, 20 μ L, and 25 μ L) on the TLC separation plate and the intensity of the resulting spots under UV light. Therefore, the purpose of this experiment was to determine the minimum sample volume required to identify aflatoxins and to investigate the relationship between volume and sensitivity/selectivity. We chose to recover AFB1 across all sample volumes from 5 μ L to 25 μ L. However, the intensity of the fluorescence spots increased linearly with the volume of the *F. vulgare* extract used. The implication is that high sample volumes are more accurate and yield higher 'readings' for aflatoxins than low volumes, indicating that they are easily detected, particularly at low contamination levels. The spot of AFB1 in TLC of a 5 μ L sample was barely visible, whereas that of a 25 μ L sample was well visualized, making it easy to determine the degree of aflatoxin contamination. Figure 4A shows the TLC with varying volumes of *F. vulgare* extract,

with band intensity increasing from 5 μ L to 25 μ L. To show more detail on the variation in band intensity, Figure 4B below shows the results when normalized to the Bradford-determined protein concentration. As this analysis shows, this distinction has implications for understanding the association between sample volume and the ability to detect certain entities. The results of the study also show that the selected *F. vulgare* extract offers detection capability as low as 5 μ L, which is very important in cases where larger spice samples cannot be analyzed due to high volumes and associated costs. Consequently, the data from this comparative analysis show that increasing the concentration of *F. vulgare* extract increases the detectability of AFB1 under TLC. Larger volumes increase the likelihood of detecting the toxin; however, even a small volume will yield measurable results, which is beneficial when the amount is small.

Quantitative Aflatoxin Concentration Analysis Across Spices

The results of this research, conducted at the quantitative levels of frequently used spices for different aflatoxins, provide information on the safety limits of spices in the context of aflatoxin contamination. Aflatoxins are toxic and carcinogenic substances produced by *Aspergillus* spp., and can be

lethal for consumers if present in food products that exceed the regulatory limits. These compounds pose serious risks and can cause serious problems for people who handle them, especially if stored in humid areas or not properly preserved. Among all the spice samples analyzed in this study, only *F. vulgare* was found to have a detectable concentration of aflatoxin, i.e., 99.72 ppb AFB1, which exceeds the permissible limit level for food items. This concentration is of profound concern as aflatoxin B1 is a known carcinogen and its presence may have dangerous health implications even if ingested sporadically, especially in children and immunocompromised persons. The high level of contamination in the *F. vulgare* suggests that the fungus could grow and that aflatoxins could develop under the storage conditions or processing methods used for the product. Factors such as humidity, poor air circulation, heat stress, or cold storage can favour fungal growth, thereby increasing aflatoxin levels. This problem may be compounded, particularly when spices are bought from farmers with small plots of land, which generally do not meet stringent quality criteria, or when foods are imported from countries with lower standards. Conversely, the other spices tested in the present investigation, including *C. cyminum*, *C. annuum*, *C. sativum*, *S. aromaticum*, *T. ammi*, *C. verum* and *C. longa*, did not show the presence of any aflatoxin in the analyses, and they were less than the permissible concentrations prescribed for spices; hence, no aflatoxin. These outcomes indicate that the spices were obtained from areas with strict quality control procedures or processed under conditions that prevented excessive fungal growth. However, the available evidence only confirms the absence of aflatoxins, even though other mycotoxins or harmful constituents may be present. However, since aflatoxins were not found, aflatoxin levels in these spices are within the legally enforceable levels set by regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), both of which have a maximum limit of 20 ppb for aflatoxin B1. The results of this study highlight the need to routinely test food commodities for aflatoxins, with spices receiving the highest priority due to varying contamination risks by country of origin and processing procedures. Moreover, the results identify potential contamination sources and underscore the need for further research to identify preventive strategies to reduce consumer risk.

Quantitative Measurement of Aflatoxin Concentration by Weight and Fitness for Consumption

In addition, a quantity-based analysis of aflatoxin concentration per gram, effective weight, and sample

was performed to evaluate the safety of each spice sample further. Sample weights, effective weights, and aflatoxin concentrations were summarized in Table 1 for each species. The effective weight accounts for factors such as moisture content and the way the spice is prepared for analysis, thereby providing a more accurate picture of the toxin's actual concentration in the analyzed spice. The results of this analysis were decisive in selecting which spices were good for human consumption, given emerging levels of aflatoxins. From the samples analyzed, *F. vulgare* was found to contain 99.72 parts per billion (ppb) aflatoxin B1 (which is above the legal limit); as such, the sample was labeled unfit for human consumption. This is because the chronic effects of exposure to such high levels of aflatoxins are well known. Especially for *F. vulgare*, stricter controls may be required, including recalls or bans on the product if such contamination occurs across most batches. However, aflatoxins were not detected in any of the other spices: *C. cyminum*, *C. annuum*, *C. sativum*, *S. aromaticum*, *T. ammi*, *C. verum*, and *C. longa*. The analysis shows that these spices meet the hygiene standards recommended by leading global health organizations. As spices are used worldwide to produce a wide range of foods and other food products, it is vital to eliminate aflatoxins from these products. Nevertheless, the presence of aflatoxins does not necessarily indicate the presence of other contaminants, including pesticide residues and heavy metals, which must also be investigated to ensure the overall quality of spices.

Effectiveness of Decontamination Methods

Upon confirmation of aflatoxin contamination in the sample, several decontamination measures were undertaken to test their efficacy in reducing or eradicating the contamination. These decontamination methods were classified into three principal groups: physical, chemical, and biological, and they represent different approaches to addressing the aflatoxin problem in food commodities. Results for each category are presented, and the research results are provided in Table 2 after analysis.

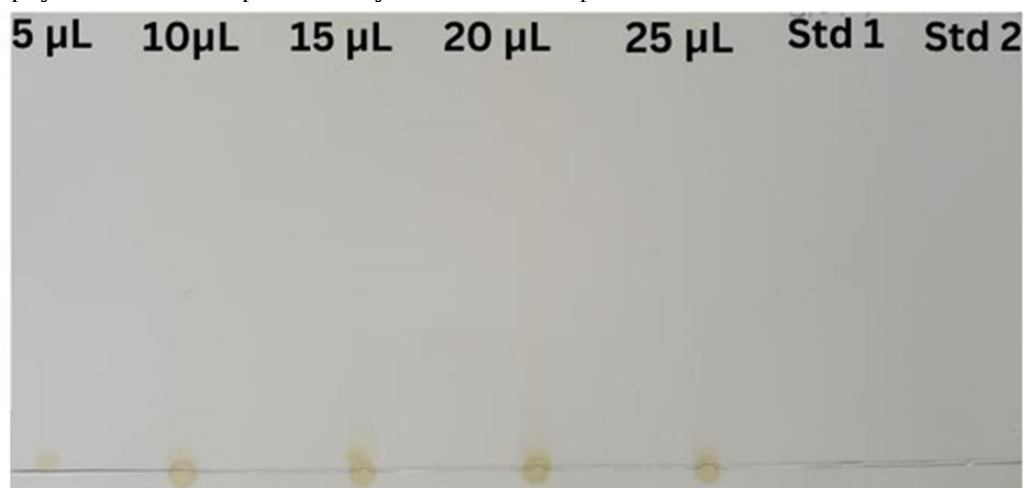
Physical Decontamination Methods

Cold and hot water treatments were among the earliest physical decontamination methods examined to reduce aflatoxin levels. Each cold-water treatment resulted in a 44.99% reduction in AFB1 levels. As a result, the process can be regarded as moderately efficient, as it is known that cold water releases aflatoxins bound to the seed surface. Nevertheless, cold water does not penetrate the matrix sufficiently to alter aflatoxins.

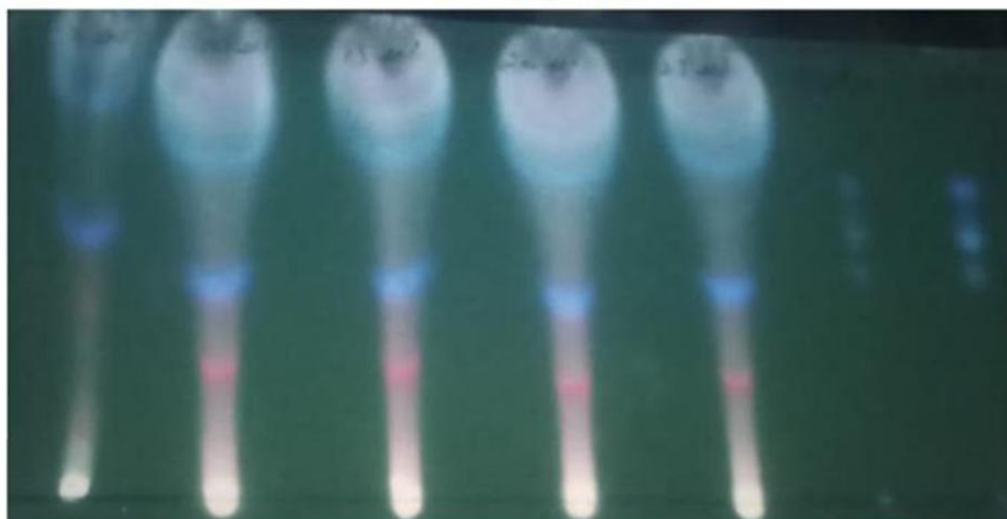
Consequently, although this method may reduce surface contamination, it is unlikely to achieve sufficient penetration to render highly contaminated products completely safe. When water is heated to

boiling, the reduction improves slightly, with a 50% decrease in aflatoxin. At the boiling point, the high temperature can lead to loss of aflatoxin molecule integrity, potentially causing denaturation or degradation. Nevertheless, the boiling process was not enough to remove all the aflatoxins from the *F. vulgare* sample, despite the results. These results showed that physical treatments provide only a limited

level of detoxification; however, they cannot remove aflatoxins from highly contaminated foods when used alone. The inadequacy of physical decontamination becomes evident when aflatoxins are still detected in deeply contaminated foods or processed foods. Accordingly, physical methods should be supplemented with chemical or biological treatments to optimize detoxification.



A. Aflatoxin concentration and its detectability at varying sample volumes



B. Variation in spot intensity based on the sample volume

Figure 4. A: TLC analysis of the detectability of aflatoxin B1 in different volumes of *F. vulgare* extract ranging from 5 to 25 µL. B: Variation in band intensity according to the sample volume of the extract showed the effectiveness of TLC in the detection of aflatoxin contamination.

Table 1. Quantitative measurement of aflatoxin concentration (ppb) in spice samples

Sr. No.	Botanical name	Sample weight (g)	Effective weight (g)	Calculation (ppb)	Detected/Not detected	Fit/Unfit
1.	<i>Cuminum cyminum</i>	50.0002	10.0000	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit
2.	<i>Foeniculum vulgare</i>	50.0034	10.0006	$\frac{5 \times 2 \times 500}{10.02804 \times 5} = 99.72$	Detected	Unfit

3.	<i>Capsicum annum</i>	50.03823	10.0076	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit
4.	<i>Coriandrum sativum</i>	50.2390	10.0478	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit
5.	<i>Syzygium aromaticum</i>	50.1402	10.0280	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit
6.	<i>Trachyspermum ammi</i>	50.1278	10.0255	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit
7.	<i>Cinnamomum verum</i>	50.1763	10.0352	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit
8.	<i>Curcuma longa</i>	50.0987	10.0197	$\frac{5 \times 2 \times 500}{10.0000 \times 0} = 0$	Not detected	Fit

Table 2. Percentage reduction of aflatoxin in positive samples by physical, chemical, and biological methods.

Sr. No.	Method	Treatment					
1.	Physical methods	Cold water			Hot water		
	Aflatoxin reduction	44.99%			50%		
2.	Chemical methods	Citric acid			Ammonium hydroxide		
	Percentage applied	5%	10%	15%	5%	10%	15%
	Aflatoxin reduction	52%	60%	64%	44.9%	60%	67%
3.	Biological methods	Moringa oil			Black seed oil		
	Percentage applied	5%	10%	15%	5%	10%	15%
	Aflatoxin reduction	55%	64%	72.5%	63%	66%	77.50%

Chemical Decontamination Methods

In the present study, decontamination protocols using citric acid ($C_6H_8O_7$) and NH_4OH at concentrations of 5%, 10%, and 15% were carried out to assess their efficacy in reducing aflatoxins. Citric acid showed reductions of 52%, 60%, and 64% at 5, 10, and 15%, respectively, indicating an increase in its capacity to detoxify aflatoxins, attributable to citric acid modifying the chemical structure of aflatoxins through hydrolysis or another mechanism, thereby reducing their toxicity. In addition, ammonium hydroxide decreased the values by 44.9% at 5%, 60% at 10%, and 67% at 15%; the highest concentration yielded the best results, indicating considerable efficiency in aflatoxin degradation, possibly due to changes in molecular configuration. Nonetheless, the efficacy of these agents in destroying aflatoxin raises questions about their use in food products. While citric acid and ammonium hydroxide are used in the preparation of soft drinks, both are corrosive chemicals, and if not properly washed off, the food product may be contaminated with hazardous chemicals harmful to consumer health. It shows that chemical treatments are likely to be effective if needed to remove aflatoxins. However, in practice, the issue of safety precautions,

such as rinsing or neutralizing residues to safe levels, needs to be addressed. Also, the potential chemical toxicity and its accumulation in the products indicate that these techniques should be applied carefully, particularly in products eaten raw or slightly processed. Thus, chemical decontamination methods could have been applied in industrial facilities as supplementary treatments to the physical ones. They could also be used for foods that may not retain chemical residues, such as those that have passed through processing or cooking.

Biological Decontamination Methods

Biological decontamination methods focused on assessing natural oils, characterized by their ability to kill fungi and detoxify, including moringa oil and black seed oil. Both oils were used at 5%, 10%, and 15% concentrations, and the levels of aflatoxins in the samples were determined. Moringa oil pharmacist concentration reductions were 55% at 5%, 64% at 10%, and 72.45% at 15%, suggesting that moringa detoxification increases with concentration. The following finding shows that moringa oil is a potent bioactive substance that can remove aflatoxin from food products. It is conceivable that the oil might bind to aflatoxin molecules, perhaps inactivating or

desensitizing them. The positive impact of Moringa oil can be attributed to its rich bioactive constituents, including antioxidants, which are likely to interfere with the aflatoxin molecule. Black seed oil, an antifungal and detoxifying natural oil, was found to be the best of the treatments tested here. When used at 5% concentration, it reduced germination capacity by 63%; at 10% by 66%; and at 15% by 77.5%. Compared with citric acid and ammonium hydroxide, black seed oil showed greater detoxification efficacy. Due to its bioactive compounds, black seed oil may be an essential supplement to facilitate aflatoxin detoxification, as thymoquinone, an active component, has potent antioxidant and anti-inflammatory activities. All the above-mentioned biological methods are natural, environmentally friendly techniques for reducing aflatoxins. In addition to being effective, they are nontoxic and can be used on food crops without containing dangerous residues on the edible parts. In addition, biological treatments help retain the spice's nutritional quality, including flavor and nutritive value, compared with chemical treatments. Moringa and black seed oils are derived entirely from natural extracts, making them easily used in the food industry and meeting the necessary standards for food safety and preservation of food quality. The decontamination trials clearly show that biological decontamination methods were

much more effective than physical and chemical treatments, as moringa oil- and black seed oil-treated samples had the lowest aflatoxin levels. These oils showed the highest reduction percentages; black seed oil offered the most significant qualitative reduction in aflatoxins, making it the most suitable candidate for large-scale aflatoxin reduction. The percentages achieved by each method also show that biological oils can reduce aflatoxin levels to a minimum. The results show that after treatment, 87.5% of the spice samples were negative for aflatoxin, while only 12.5% were slightly contaminated. These results, therefore, illustrate the need for greater efficacy and specificity in decontamination processes, especially for spices from uncontrolled markets, where the risk of contamination with raw aflatoxins is high. The conclusions are that developing biological decontamination approaches could shift the trend in food safety, generating a strong demand, especially in the seasoning market, where the threat of aflatoxin contamination is high. These are eco-friendly, safe for consumers, and highly efficient; hence, they should be used as the best way to mitigate aflatoxin risks in food. Moreover, I observed that their use in oil-based products can improve quality, prevent contamination, and enhance public health, as they do not affect the nutritional value or taste of the food.

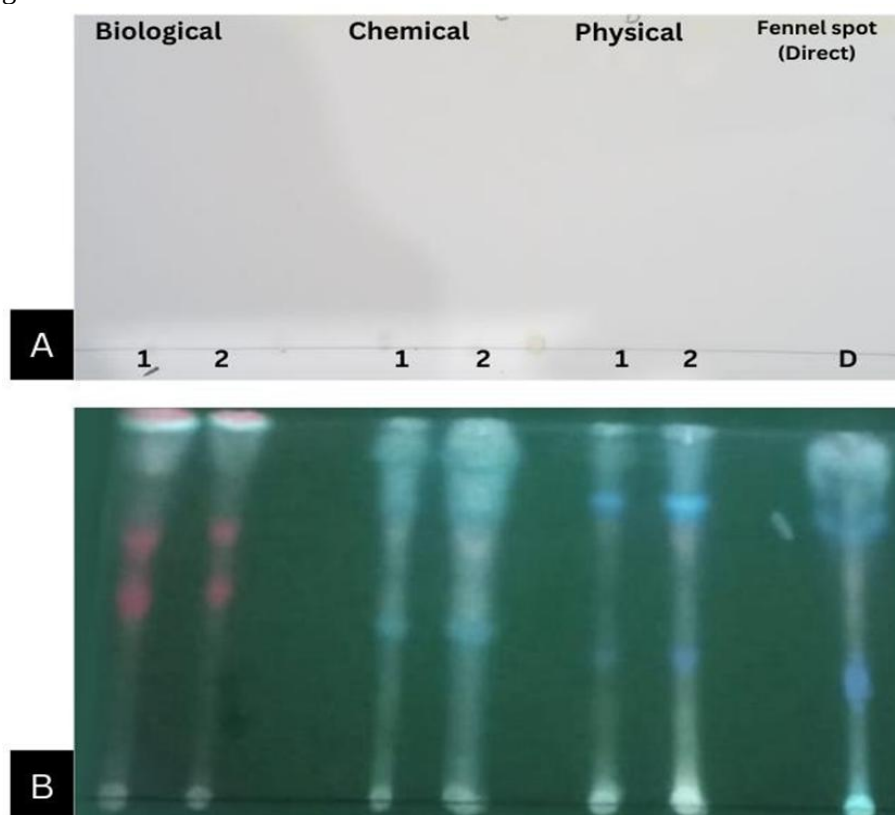


Figure 5. Detoxification strategies adopted to decontaminate aflatoxins and to ensure safety. Two treatments were used for each method. For biological methods, 1) Moringa oil and 2) Black seed oil, for chemical methods, 1) Citric acid and 2) Ammonium hydroxide, and for physical methods, 1) Cold water and 2) Boiled water.

Therefore, it might be possible to reorient the focus to a few biological methods, such as moringa oil and black seed oil, as a significant strategy to improve food safety in the global spice trade. These methods are environmentally friendly and effective in addressing the aflatoxin issue, which hampers food production and thus align with people's rising demand for healthful foods that contain no chemicals.

Quantitative Moisture Analysis and Statistical Findings

Quantitative moisture analysis of a variety of spice samples showed that moisture content varied significantly across samples, a finding that is crucial for assessing the risk of aflatoxin development (Table 3). Using a quick moisture analyzer, we determined that spices with high moisture content are significantly more vulnerable to contamination by aflatoxin-producing fungi. Among the spices evaluated, *F. vulgare* had the highest moisture content, measured at 19.78 %. This increased moisture content makes *F. vulgare* especially vulnerable to fungal proliferation and the resulting aflatoxin contamination, particularly under ambient storage conditions in conventional storage systems. After *F. vulgare*, *C. annuum*, and *S. aromaticum* showed moisture contents of 10.78 and 11.68%, respectively; these values are lower than those of *F. vulgare*, but represent a moderate risk of contamination. On the other hand, *C. longa* showed the lowest moisture content (5.78 %), suggesting that *C. longa* is less susceptible to aflatoxin growth, even under environmental conditions that may favor fungal growth in other spices. These results show that the moisture content of spices is quite variable and directly affects contamination potential, thereby requiring additional preventive measures for high-moisture varieties.

A one-way analysis of variance (ANOVA) was used to assess differences in observed moisture content and to evaluate their statistical significance. The goal was to determine whether the differences in moisture levels between the spices were statistically significant. For the published data, the analysis of variance (ANOVA) showed an F statistic of 4.26 and a p-value less than 0.05 for all spice samples. These findings show that there were statistically significant differences in moisture content among the spices, thereby rejecting the null hypothesis that all spices have similar moisture-retention properties. The observed variation suggests that some spices, especially those with higher moisture content, are more likely to support the growth of aflatoxin-producing fungi and thus are at higher risk of

contamination. Therefore, spices with a high moisture content require special storage procedures to reduce the risk of aflatoxin growth.

Furthermore, Tukey's honestly significant difference (HSD) test was conducted as a post hoc analysis to identify specific pairs of spices with substantial differences in moisture content. The Tukey HSD indicated significant differences in moisture content between *F. vulgare* and *C. cyminum*, and between *C. longa* and *C. cyminum* (p-value = 0.01). This result confirms that *F. vulgare* is more susceptible to aflatoxin contamination due to its high moisture retention capacity. Likewise, *C. annuum* showed much higher moisture levels than *C. cyminum* and *C. longa*, highlighting the need for better moisture control for spices that tend to retain moisture. These comparisons provide a complete risk picture for each spice, with *F. vulgare* and *C. annuum* being more prone to contamination due to their higher moisture content, compared to less-prone spices such as *C. cyminum* and *C. longa*. The objective of studying possible interactions between the spice type and the duration of moisture measurement was addressed using a two-way Analysis of Variance (ANOVA). The analysis sought to determine whether the moisture content of individual spices was affected by the time required to achieve a stable measurement, and whether any unique properties affect moisture retention. The two-way analysis of variance yielded an F-value of 5.34 and a p-value of 0.02, indicating a statistically significant interaction between spice type and measurement duration. This interaction has shown that some spices not only vary in their moisture content but also in how they respond to measurement duration, which is determined by their structural and physical properties. Accordingly, the results highlight the need to use specific analytical techniques when evaluating spices with high water content, as measurement time may affect moisture stability and thereby increase the risk of contamination. Based on moisture content data and statistical analyses, recommendations for moisture control were developed for each spice. *F. vulgare*, with the highest moisture content, was considered a high-risk spice requiring stringent moisture-control measures to prevent aflatoxin growth. *C. annuum* and *S. aromaticum* are also worthy of moderate monitoring because of their relatively high moisture content. In contrast, *C. longa* and *T. ammi*, with the lowest moisture levels, present minimal risk for fungal contamination and therefore require only basic moisture monitoring to maintain safe storage conditions.

Table 3. Moisture content analysis and statistical results for spices.

Botanical Name of Spice	Weight (g)	Time Taken (min)	Moisture Content (%)	ANOVA F-value	ANOVA Significance (p-value)	Tukey's HSD (Compared Pairs)	Two-Way ANOVA F-value	Two-Way ANOVA Interaction (p-value)
<i>Cuminum cyminum</i>	1.0009	6	7.35	4.26	0.03	p=0.02	5.34	0.02
<i>Foeniculum vulgare</i>	1.0019	18	19.78	4.26	0.02	p=0.01	5.34	0.02
<i>Capsicum annum</i>	1.0001	16	10.78	4.26	0.01	p=0.01	5.34	0.02
<i>Coriandrum sativum</i>	1.0054	5	8.21	4.26	0.03	p=0.03	5.34	0.02
<i>Syzygium aromaticum</i>	1.0881	14	11.68	4.26	0.02	p=0.02	5.34	0.02
<i>Trachyspermum ammi</i>	1.015	7	6.67	4.26	0.01	p=0.01	5.34	0.02
<i>Cinnamomum verum</i>	1.0056	13	7.56	4.26	0.04	p=0.01	5.34	0.02
<i>Curcuma longa</i>	1.0012	10	5.78	4.26	0.03	p=0.01	5.34	0.02

Sensory and Nutritional Quality Assessment Post-Detoxification

Studies on sensory and nutritional changes in detoxified spices after the chemical and biological treatments showed that the modifications least affect the acceptability and nutritional value of spices. Sensory analysis results using a 5-point hedonic scale show that chemical and biological treatments of cottage cheese yielded comparable results for color, aroma, flavor, and texture, with mean values ranging from 4.2 to 4.5. Among the treatments, the biological method, especially with black seed oil, was the best, retaining the spices' sensory characteristics with scores closer to the maximum (4.5). These results indicate that biological detoxification strategies, especially black seed oil treatment, are most effective in preserving the organoleptic properties of the spices after detoxification. To negate these effects and

evaluate the nutritional value of detoxified spices, the high protein and fat content of the spices was estimated before and after detoxification. Using the Kjeldahl and Soxhlet extraction methods, this study concluded that detoxification did not reduce the nutritional value of the recommended spices. Protein and fat retention were at par, with a range of 95-98% across all treatments. This outcome is illustrated in Figure 5, which presents the density of sensory scores for chemical and biological detoxification methods. As shown in Figure 6, the post-treatment difference in sensory scores is minimal, and both methods maintain the acceptability of the spices. Moreover, the density plots reveal that, with biological treatments, especially with black seed oil, the distribution of the sensory properties near the higher scores for color, aroma, and flavor shows greater density in the center.

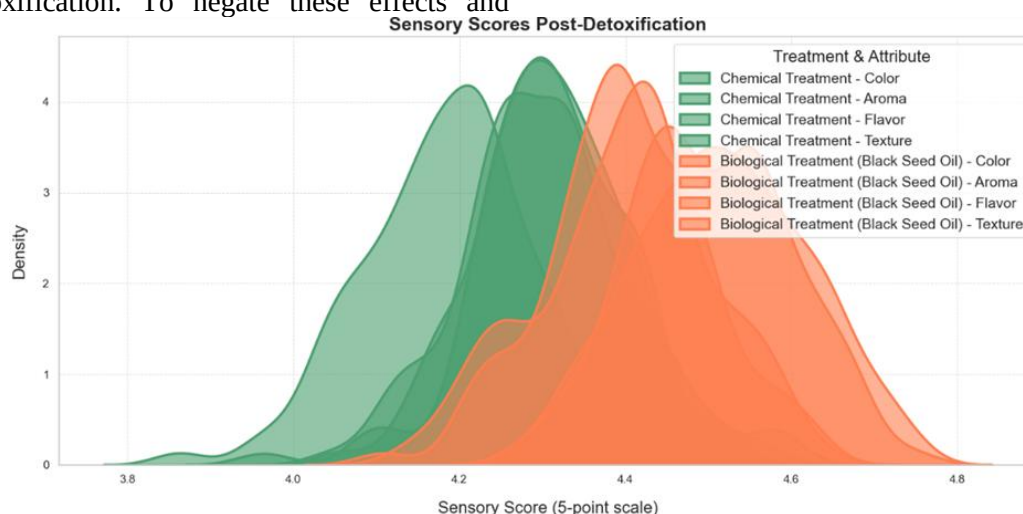


Figure 6. Sensory scores for color, aroma, flavor, and texture of detoxified spices following chemical and biological detoxification treatments.

These results highlight the effectiveness of the tested detoxification methods, especially the biological methods, and recommend their use to make spices safe for human consumption and to ensure that aflatoxin levels do not affect their nutritional value. The sensory analysis, together with the nutritional study, supports the applicability of the detoxification treatments as valid alternatives for ensuring the quality of affected spices.

DISCUSSION

Results from a survey indicated a wide range of moisture content and aflatoxin contamination levels in unbranded spice samples collected from various markets in Lahore. The seven unbranded spice samples tested for aflatoxin contamination contained <90 ppb of aflatoxins, except for one sample containing 99.72 ppb of AFB1 in *F. vulgare*. However, we found that none of the test samples contained detectable levels of aflatoxin (Alinezhad et al., 2011; Kedia et al., 2015), consistent with earlier studies showing higher contamination levels in *F. vulgare* than in other unbranded spices. Characterization of AFB1 using thin-layer chromatography (TLC) confirmed its presence in *F. vulgare* and demonstrated that TLC is an accurate method for detecting AFB1 (Abbas et al., 2021). The remaining spices were not contaminated with aflatoxins, as shown in the present study (*C. cyminum*, *C. annum*, *C. sativum*, *S. aromaticum*, *T. ammi*, *C. verum*, and *C. longa*), and supported by the work of Tilak et al. (2024), who also showed that appropriate drying and storing of spices can help to prevent the growth of fungi. *F. vulgare* has a high level of AFB1; therefore, there is a serious risk to public health due to AFB1's potential carcinogenicity (hepatocellular carcinoma) and immunotoxicity (Al Ayoubi et al., 2021). Our results align with the WHO's (2018) suggestion, indicating that increased regulation and ongoing monitoring should be established for unbranded spice products.

In a comparison of detoxification techniques, cold and boiling water were the least effective at reducing aflatoxin B1 (44.99% and 50%, respectively). Thus, previous studies have noted that their main use is to remove surface contamination (Peles et al., 2019; Ryan et al., 2014). Chemical methods yielded the most effective results: under formaldehyde treatment, ammonium hydroxide (15%) reduced AFB1 levels by 67%, and citric acid by 64%. These results are similar to those of Yang et al. (2019), Chen et al. (2008), and Zhang et al. (2019), who reported that structural degradation of AFB1 occurs under both acidic and alkaline conditions. Residues from chemical methods pose implementation issues (Mohiuddin et al., 2019; De Corato et al., 2019). However, natural oil sources derived from black seed and moringa showed greater-

than-expected efficacy (77.5% and 72.45%, respectively). These results also support earlier studies of the antifungal and detoxifying properties of natural oils (Achar et al., 2020; Ahmad et al., 2022). The use of these methods provides benefits such as environmental safety, the absence of toxic residues, and the preservation of product quality (Ezzatpanah et al., 2022). Overall, these findings confirm *F. vulgare* spices as a high-risk category and further confirm that biological methods are the most effective and environmentally sustainable means of reducing aflatoxin levels. Since these techniques have been reported as effective, future work should include additional research to scale up methods for routinely evaluating their long-term effects and utility within food systems.

CONCLUSION

This research identifies aflatoxin contamination in several spices commonly used in food and examines methods for decontaminating them. From eight spices or condiments, the following spices were identified and purchased from different markets: *C. cyminum* procured from First Capital Super Store, *F. vulgare* from A One Super Store, *C. annum*, and *C. sativum* from Osh Meta Foods, and *S. aromaticum* from Seasoning. They were kept in tightly sealed containers at room temperature (22-25°C) to confer the above characteristics. Thin-layer chromatography was used to detect aflatoxin contamination, and only 1 of 8 samples tested (12.5%) was found to contain aflatoxins. The contaminated spice was *F. vulgare*, with a very high level of AFB1 at 99.72 ppb, exceeding the safe limit of 20 ppb for aflatoxins in spices. As a result, this particular sample is not fit for human consumption. Among the remaining seven samples, *C. cyminum*, *C. annum*, *C. sativum*, *S. aromaticum*, *T. ammi*, *C. verum*, and *C. longa* all tested negative for aflatoxins; therefore, they are safe for human consumption.

This study also focused on various decontamination methods to reduce aflatoxin levels in contaminated spices. Physical processes using cold and heated water resulted in modest reductions in aflatoxin levels, with observed levels of 44.99% and 50%, respectively. While these approaches achieved moderate detoxification, complete elimination of aflatoxin was not achieved. Decontamination with citric acid and ammonium hydroxide was more effective than the above. 25g/L of citric acid lowered aflatoxins by 52%, 50g/L lowered aflatoxins by 60% whereas 75g/L of citric acid lowered aflatoxins by 64%. Likewise, results for ammonium hydroxide showed decreases of 44.9% at 5%, 60% at 10%, and 67% at 15%. These chemical treatments proved more effective at higher concentrations, though the issue of chemical residues

on spices must be considered. The study also assessed the biological decontamination process, and among the results obtained, Moringa oil and Black Seed oil were relatively effective. Moringa oil reduced aflatoxin by 55% at 5%, 64% at 10%, and 72.45% at 15%; BS oil was the most effective method for reducing aflatoxin.

From the analysis of moisture content, the result indicated that the highest value of moisture was selected for *F. vulgare* in the range of 19.78%, the *C. annum* value was 10.78%, while for *S. aromaticum*, the value recorded was 11.68%; the two lowest values were for *C. longa*, 5.78%, and ajwain at 6.67%. From the results below, we determined that the moisture content of spices such as *C. cyminum* and *C. longa* was lower, whereas the highest values were observed for *F. vulgare* and *C. annum* ($p = 0.01$). The One-Way ANOVA test yielded $F = 4.26$, significant at $p = 0.03$; thus, the moisture content in spices is significantly different. As with enzyme activity, Two-Way ANOVA showed a significant interaction between spice type and measurement time for moisture content (F -value = 5.34, p -value = 0.02). The incidence of aflatoxin contamination is higher in spices with high moisture content, such as *F. vulgare* and *C. annum*, which require moisture control during storage.

Regarding the comprehensive assessment of sensory quality, the hedonic scale scores for the detoxified spices ranged from 4.2 to 4.5, indicating that the detoxification treatments had a negligible effect on the spices' sensory characteristics. Among the four decontamination methods, the biological method using black seed oil achieved the highest score of 4.5, identical to that of the control sample. The results of the nutritional analysis of these foods are as follows: Protein and fat retention ranged from 95% to 98%, indicating minimal nutrient loss during detoxification. These data highlight the potential of biological methods, especially Black Seed oil, for detoxifying spices with minimal changes in their nutritional values. The results further indicate that, with appropriate moisture control and effective detoxification methods, aflatoxin contamination of spices can be minimized, thereby ensuring their safety for human consumption.

Authors Contribution

Maryum Zainab and Samee Ullah Butt: Data curation, investigation, methodology, writing- review & editing. Umer Majeed, Aqiba Ishfaq, Areeba Mukhtiar and Rana Muhammad Hammad: Validation, formal analysis, writing original manuscript. Neeha Ishfaq, Shoaib Habib and Shanza Monis: Visualization, Projects administration, writing- review & editing. Muhammad Shah Jahan: Software, writing- review & editing. Azeem Sharif: Supervision, writing- review &

editing. Naureen Anwar: Conceptualization, writing- review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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