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Aflatoxin in commonly used spices collected from local markets of Karachi, Pakistan

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ABSTRACT

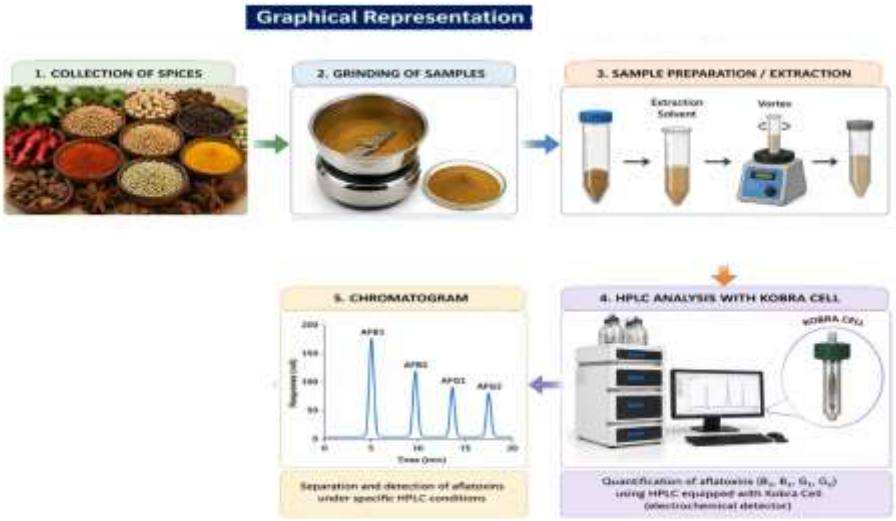
This survey was carried out to evaluate the occurrence of total aflatoxins (AFs; B1+B2+G1+G2) in unpacked spices and herbs of five different regions of Karachi, Pakistan namely Nazimabad, Gulshan-e-Iqbal, Jona, Korangi, and Empress Market. The occurrence and concentration levels of aflatoxin contamination in unpacked spice samples obtained from Karachi's main markets were assessed using the HPLC technique. 78 (86.66 percent) of the 90 samples tested positive, with the majority of the contamination falling between 1 and 10 µg kg^{-1} and none exceeding 10 µg kg^{-1}. The highest mean aflatoxin levels among spices were found in dried ginger (3.36 ± 0.45 µg kg^{-1}) and black caraway (3.12 ± 0.38 µg kg^{-1}), whereas the lowest concentrations were found in turmeric (1.42 ± 0.98 µg kg^{-1}) and fenugreek (1.45 ± 0.63 µg kg^{-1}). In terms of markets, Gulshan-e-Iqbal had a lower positivity rate (33%), while Empress, Jona, Nazimabad, and Korangi showed 100% contamination. Gulshan-e-Iqbal had the lowest mean level (0.39 ± 0.24 µg kg^{-1}), while Korangi Market had the highest (4.45 ± 0.98 µg kg^{-1}), followed by Empress Market (3.89 ± 0.64 µg kg^{-1}). The statistical analysis was used for statistical analysis; all data were expressed as mean ± standard deviation (SD), and $p < 0.05$ was deemed statistically significant. The results show widespread aflatoxin contamination overall, emphasizing the necessity of better handling, storage, and monitoring procedures to lower possible health risks.

Keywords: Spices, Aflatoxin, HPLC, Mycotoxin contamination, Kobra cell derivatization

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INTRODUCTION

Spices are mainly utilized to enhance the flavor, color, or preservation of food. They may also consist of dried seeds, fruits, roots, barks, or plant materials. The popularity of spices has risen considerably their distinctive attributes, such as therapeutic properties, culinary benefits, and unique tastes, hues, and aromas, make them highly desirable and commonly used as vital ingredients in cooking. Red and black chilies, fenugreek, turmeric, black seed, bay leaves, fennel, garlic, clove, cinnamon, coriander, cardamom, cumin, pepper, and white cumin are some of the spices that are commonly used in the Asian region. Toxic molds can infect spices before and after they are harvested, dried, processed, packed, stored, and shipped. Due to unfavorable assortment conditions like a negligent production process, inappropriate collection settings, and inadequate drying times, spices are susceptible to microbial attacks from a wide range of sources(Banerjee & Sarkar, 2003; Cokosyler, 1999). Scientists have discovered more than 300 mycotoxins, but those with teratogenic, mutagenic, and carcinogenic qualities have received the most attention(Jallow et al., 2021).

According to (Martins et al., 2001), Spices that are typically produced in countries with high temperatures, tropical humidity, and regular rainfall are conducive to the growth of mycotoxigenic fungi and, ultimately, the production of mycotoxins.

Mycotoxins are secondary fungal metabolites that are produced by Aspergillus, Fusarium, Pecicillium, and other toxic species. Aflatoxins (AFs), ochratoxins, zearalenone, citrinin,and trichothecenes are a category of mycotoxins that are captivating because to their potential for harm (Ismaiel & Papenbrock, 2015). The most prevalent mycotoxins are called AFs. According to (Varga et al., 2011), AFs are carcinogenic, mutagenic, immunosuppressive, hepatogenic, and neoplastic. B1, B2, G1, and G2 are the highly toxic AFs. (Sudini et al., 2012) state that AFB1 has the highest degree of toxicity and is often associated with sever hepatotoxicity and hepatocarcinogenicity. The production of AFs is greatly influenced by a number of factors, such as temperature, moisture content, water activity, pH, and oxygen concentration. (PRATIWI et al., 2015).

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Aflatoxins (AFs) have been detected in a number of agricultural commodities, such as rice, maize, peanuts, groundnuts, cotton seed, chicken feed, and its components. ((Asghar, Ahmed, et al., 2016; Bircan & Koç, 2012). Aflatoxin contamination in spices is a serious issue since Pakistan's climate, agriculture, storage, and transportation conditions are conducive to aflatoxigenic growth. the production of AFs, and finally, molds. Unfortunately, the cleanliness of Pakistan's marketplaces, particularly in Karachi, is quite poor. Most residents of Karachi come from lower socioeconomic classes and are unaware that spices may contain AFs. The unpacked ground spices create an ideal environment for the growth of fungus when stored for a long time in a damp, humid environment. Furthermore, loose-packaged compound spices from bazaars and wholesale markets could easily become contaminated by dust, waste water, and animal or human waste. (Ajmal et al., 2022)

Furthermore, it is believed that the hygroscopic nature of spice powder and a warm, humid environment are highly favorable for the development of toxic fungi and the formation of AFs. Many countries, including Pakistan, have put strict guidelines into place to reduce the negative consequences of AFs contamination in spices and their constituents. The maximum limits (MLs) for total AFs and AFB1, for instance, are 10 μg kg^{-1} and 5 μg kg^{-1}, respectively, according to the European Union (EU)(Commission, 2006). However, The USA (FDA and FAO) set the ML for total AFs at 20 μg $kg^{-}1$ ((FDA), 2000). Several steps have been taken by the Pakistan Standard Quality Control Authority (PSQCA) to reduce the risk that mycotoxins, especially AFs, pose to human and animal health. Pakistan currently complies with US and EU mycotoxin contamination regulations. Several authors have discovered that a variety of food items are contaminated with AF. ((Asghar, Iqbal, et al., 2016; Aydin et al., 2011; Trombete et al., 2014).Nonetheless, there are still few details available regarding the rules and monitoring of AFs in spice samples sold in local markets, in Karachi, Pakistan. This study was conducted to assess the level of AF contamination in commonly used spices (unpacked) food items in order to increase awareness of the epidemiological effects of contaminated spices. However, as a developing country, we find it difficult to control the levels of various pollutants, especially aflatoxins.

MATERIAL AND METHOD

Sample Collections

From February to July 2025, a total of 90 samples of unpackaged spices were gathered from retail stores, open bazaars, and local markets in five different regions of Karachi, Pakistan. The spices included Coriander, Cinnamon, Dried ginger, Black caraway, Turmeric, Fenugreek. The fact that AFs are unevenly distributed across the commodity has plenty of verified data. To create homogeneous samples, an approach outlined in AOAC method no. 977.16 (International, 2002) was used. To put it briefly, 500 of each sample were collected and thoroughly mixed for ten minutes. Each sample was ground using a sample grinder to produce a consistent and representative sample. To find the AFs, 25 g of each sample was collected. All milling samples were kept in sealed polyethylene bags at -20°C until further examination.

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Chemical and reagents

B1(Art. #002017, 2µg/ml), B2(Art. #002018, 0.5µg/ml), G1(Art. #002019,2 µg/ml), G2 (Art. #002020, 0.5µg/ml) obtained from Biopure, PBS (phosphate buffer saline) tablets with a pH of 7.3±0.2 were purchased from Oxoid in Vienna, Austria (UK), Acetonitrile, methanol, HNO3, and KBr from sigma Aldrich.

Instrumentation

The HPLC system was made up of a pump, auto sampler, and detector (FLD) with RP C18 column (5µm, 250×4.0mm) purchased from Germany, Kobra cell was obtained from Lanark shire., UK, Aflatoxin immune affinity column was purchased from Roomer lab, Austria, a blender which is explosion proof was obtained from New York, USA

sample preparation for aflatoxin analysis

500 g of each sample were collected and aggressively blended for 10 minutes. To obtain a homogeneous and representative sample, each sample was milled using a sample grinder. For AFs determination, 10 g of each sample was extract before the further investigation, all milling samples were stored in sealed polyethylene bags at 20°C.

Using an explosion-proof blender, 10g of each powdered sample was mixed for 2 minutes at a speed of 6000 rpm in 100 ml of ACN and water (60:40) solution. The mixed extracts were collected into clean flasks after being filtered with Whatman No. 4 filter paper.

Immunoaffinitycolumns were used for separation and Afs clean-up

2 ml of each filtered extract were diluted in 48 ml of phosphorous buffer saline with pH 7.4 using this method. At a fluid passing rate of 1 drop/s, the diluted extract (50 ml) was sprayed onto the immune affinity columns. The IACs were promptly dried by flowing air for 15 seconds after being rinsed with PBS 20ml at a fluid passing rate of roughly 6 ml/min. 1.5 ml of methanol analytical grade and 1.5 ml of H2O (deionized) were used to elute AFs in amber vials. The extract was then introduced into the HPLC-FLD system after being filtered over a 0.45 µm membrane.

HPLC-FL analysis with Afs derivatization

A Kobra Cell was used to post derivatization of AFs, and the HPLC system was used to quantify them after that. The mobile phase was ACN: MeOH: H2O (1:1:4) in isocratic mode at 1 ml/min with 119 mg/l of KBr and 154 µl/l of HNO3 (65%). For each standard or sample, there was a 100 µl injection volume. 40°C was kept as the column temperature. The wavelengths for excitation and emission were set at 365 and 435 nm, respectively. The Kobra Cell current source was kept at 100 A. Within a run time of roughly 10 to 20 minutes, all AFs were successfully addressed.

Validation of procedures and quality control

Each sample's analysis was carried out three times to ensure the validity of the analytical process.

Additionally, the HPLC method's linearity, LOD, LOQ, as well as recovery studies,

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were all validated. The method was adapted from (Asghar, Zahir, et al., 2016) with minor modifications

Linearity

The linearity of the approach was assessed using the coefficient of determination (R2). Stock standard solutions were used to generate a number of working standard solutions (total AFs = 0.05, 0.10, 0.50, 1.0, 2.0, 2.50, 5.0, 10, 20, 40 ng/ml). These solutions were then made in methanol and H2O (1:1). Area was plotted separately against concentration to create calibration curves. Using the regression equations, R2 values were determined (Lane 2015). Over the tested range, the calibration curves for AFB1, AFB2, AFG1, and AFG2 were linear with R2 in the range 0.9991−0.9997.

Quantification and the limit of detection (lod) (loq)

The calibration curves at the concentration with a peak area ratio of signal to noise of at least 3 and 10 were used to calculate the LOD and LOQ of the employed method. The obtained values satisfy the maximum limits as regulated by EU, USA and Pakistan.

Precision

The control sample was used to calculate the HPLC method's accuracy and precision precision (RSD%).RSD percentages showed good method performance after homogenized samples (n = 20) were analyzed. 95% confidence level (Commission, 2006)

Recovery

Spiked samples were used to assess the method's effectiveness. In brief, AFs standards at 0.5, 2.5, and 10 µg kg−1 were added to 25 g of blank sample (previously tested using ELISA) at least an hour prior to analysis. HPLC was finally used to quantify the AFs levels in spiked samples. According to the Codex Alimentarius Standard(Commission, 1995) and the AOAC, average recoveries were between 93.8 and 97.1 percent, which is within acceptable ranges.

Statistical analysis

the Student's t-test was used for statistical data analysis, with $p < 0.05$ serving as the minimal level of significance. Every experiment's data was presented as the mean ± standard deviation (SD).

Table 1 Validation parameters for the HPLC determination of AFB_1, AFB_2, AFG_1 and AFG_2

Validation parameters	AFB_1	AFB_2	AFG_1	AFG_2	Total AFs
LOD	0.03	0.05	0.06	0.04	0.18
LOQ	0.1	0.166	0.2	0.133	0.6
Correlation coefficient (R2)	0.9995	0.9992	0.9997	0.9994	0.9995
Recovery	92.2-97.3	94.3-97.8	92.3-95.1	95.3-98.3	95.1-97.2

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Table 2 Total aflatoxins in unpacked spices samples collected from various local markets of Karachi, Pakistan,

Samples	No. of samples	Positive samples (n%)	sample concentration ($\mu g\ kg^{-1}$) in range			Ranges ($\mu g\ kg^{-1}$),	Average ($\mu g\ kg^{-1}$)
			1<	1-10	>10		
Coriander	15	12 (80)	0	15	0	1.24-5.60	2.73 ± 0.46
Cinnamon	15	15 (100)	0	12	0	0.98-4.80	2.09 ± 0.85
Dried Ginger	15	12 (80)	0	15	0	1.17-9.01	3.36 ± 0.45
Black Caraway	15	15 (100)	0	15	0	1.21-9.12	3.12 ± 0.38
Tumeric	15	12 (80)	3	12	0	0.49-2.71	1.42 ± 0.98
Fenugreek	15	12 (80)	3	12	0	0.12-3.48	1.45 ± 0.63
Total	90	78 (86.66)	6	81	0	0.12-9.01	2.36 ± 0.62
RSD%		1.81	1.32		1.18	0.54	1.51

RESULT AND DICUSSION

Table 3 Total aflatoxins in composite spices samples per location. Measurements in triplicate, reported as mean ± SD.

Location	No. of samples	Positive samples (n%)	sample in concentration range ($\mu g\ kg^{-1}$)			Ranges ($\mu g\ kg^{-1}$)	Average ($\mu g\ kg^{-1}$)
			1<	1-10	>10		
Empress Market	18	18 (100)	0	18	0	1.17-9.12	3.89 ± 0.64
Jona Market	18	18 (100)	6	12	0	0.12-5.45	1.65 ± 0.44
Gulshan e Iqbal Market	18	6 (33)	0	6	0	1.17-1.21	0.39 ± 0.24
Nazimabad Market	18	18 (100)	0	18	0	1.10-2.41	1.43 ± 0.12
Korangi Market	18	18 (100)	0	18	0	1.44-9.01	4.45 ± 0.98

Table2 summarizes that the 78 samples (86.66 percent) of the 90 unpacked spice samples that were gathered from Karachi's local markets tested positive for total aflatoxins. No sample exceeded 10 $\mu g\ kg^{-1}$, indicating moderate contamination levels, while the majority of contaminated samples (81) fell between 1 and 10 $\mu g\ kg^{-1}$. Turmeric and fenugreek had lower mean concentrations, while dried ginger and black caraway had comparatively higher mean concentrations (3.36 ± 0.45 and 3.12 ± 0.38 $\mu g\ kg^{-1}$, respectively). Aflatoxin concentrations ranged from 0.12 to 9.01 $\mu g\ kg^{-1}$, with an overall mean of 2.23 ± 0.62 $\mu g\ kg^{-1}$.

Table 3 summarizes the presence of total aflatoxins in commonly used spice

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samples obtained from various Karachi markets. 18 samples were taken from each market location,90 spice samples that were examined. The findings showed that aflatoxin contamination varied significantly between the markets under study. All 18 of the samples taken from Empress Market tested positive for aflatoxins, indicating 100% contamination. The average concentration was 3.89 ± 0.64 µg kg^{-1}, with a range of 1.17 to 9.12 µg kg^{-1}. In a similarly, samples from Jona Market showed 100% positivity despite relatively lower levels of contamination. 6 samples in this market had aflatoxin levels less than 1 µg kg^{-1}, and twelve samples had concentrations between 0.12 and 5.45 µg kg^{-1}, with an average of 1.65 ± 0.44 µg kg^{-1}. In contrast, samples taken from Gulshan-e-Iqbal Market had a lower contamination frequency, with only 6 (33%) positive samples. The concentrations were ranging from 1.17 to 1.21 g kg1, with an average level of 0.39 0.24 µg kg^{-1}. However, Nazimabad Market showed a complete contamination, exhibiting aflatoxin concentrations ranging from 1.10 to 2.41 g kg^{-1} and a mean value of 1.43 0.12 µg kg^{-1}. All samples tested positive and aflatoxin levels ranged from 1.44 to 9.01 µg kg^{-1}, with an average value of 4.45 0.98 µg kg^{-1}. The results showed that aflatoxin contamination is widespread in composite spices sold in local markets, although most detected levels remained within the permissible safety limits. The highest mean concentration was recorded in samples from Korangi Market. Regulations by the European Commission set the maximum permissible limit for total aflatoxins in spices at 10 g kg1 and the limit for aflatoxin B1 at 5 µg kg^{-1}. All of the samples analyzed in this study were within the regulatory limit for total aflatoxins, which means that the levels of contamination observed in this study are within internationally accepted safety limits.

Aflatoxin contamination of spices is frequently linked to Aspergillus species, which can grow in environments with high humidity, temperature swings, and improper storage procedures. Traditional open markets, where spices are frequently kept in bulk containers and exposed to air and moisture for extended periods of time, frequently experience these environmental conditions. Fungal growth and the production of toxins may also be facilitated by improper drying during post-harvest handling(Naz et al., 2022; Neogi et al., 2024; San Phyo et al., 2024). The current study's conclusions are in line with earlier research carried out in other nations. For instance, research on spices and herbs in European markets revealed average total aflatoxin levels of about 6.3 µgkg^{-1}, which stayed below the European regulations maximum allowable limit of 10 µg kg^{-1}(Gialluisi et al., 2025). Similar to this, studies on organic spices showed that a considerable percentage of samples had detectable levels of aflatoxins, underscoring the pervasiveness of these toxins in spice commodities across the globe(Prelle et al., 2014).

Numerous studies have documented the widespread presence of aflatoxins in spices as a result of Aspergillus species-related fungal contamination. Spices are extremely vulnerable to mycotoxin contamination during production and storage, as evidenced by a study that examined organic spices and discovered that aflatoxin B_1 was found in many spice samples, with several exceeding regulatory limits(Tosun & Arslan, 2013). Similarly, an analysis of spices sold in Qatar revealed that aflatoxins were found in a number of spices, including black pepper, turmeric, and chili powder, and that fungal isolates like Aspergillus flavus, Aspergillus nomius, and Aspergillus niger were

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frequently found (Hammami et al., 2014; Tavakol Noorabadi et al., 2026). Additionally, because high temperatures and humidity encourage fungal growth and toxin biosynthesis, researchers have highlighted that spices grown in tropical and subtropical regions are especially vulnerable to aflatoxin contamination(Ahmed & Asghar, 2021; AJEWOL, 2023).

CONCLUSION

Frequent consumption of AFs-contaminated spices is linked to serious health risks for consumers because these spices are an essential component of Asian cuisine. In order to make sure that AFs are not present at levels that could endanger public health, it is crucial that Pakistani regulatory authorities regularly look into and monitor AFs contamination in composite spice samples. To reduce fungal growth and, eventually, the production of AFs, harvesting, drying, storage, and transportation conditions should also be carefully regulated. The best way to reduce AFs contamination in agricultural commodities is to stop fungal growth at the farm level. Aflatoxigenic mold and AF content in postharvest commodities should be inactivated using certain techniques. It is recommended that consumers purchase food products from reputable and trustworthy retailers, store them in cool, ventilated spaces, and discard any unclean, opened, or damaged packaging.

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