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# ROLE OF PHYSIOCHEMICAL PARAMETERS ON THE EXTENT OF AFLATOXINS IN CURRY/CHILI POWDER

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**Abstract**

*The present investigation aimed to conduct a comprehensive analysis of curry/chili powder and its constituent ingredients to detect the presence of aflatoxins (AFs). Additionally, the study sought to examine various physicochemical factors like moisture or citric acid that could potentially have a significant role in mitigating aflatoxin levels in curry powder. The mean concentrations of aflatoxins $B_1$ ($AFB_1$) in ground chilies obtained from Jehlum, Jhang, and Faisalabad were 69.05 ± 6.44, 31 ± 10.02, and 65 ± 5.79 μg $kg^{-1}$ with standard deviation, respectively. The mean concentrations of $AFB_1$ in curry powder obtained from Jehlum, Jhang, and Faisalabad were 39 ± 10.07, 27 ± 8.82, and 35 ± 10.04 μg $kg^{-1}$ with standard deviation, respectively. The present study also revealed that in the presence of moisture at a level of 15 % showed a significant impact on the concentration of aflatoxin in red chili powder, resulting in a range of 8 - 13 %. Similarly, in the case of curry powder, the same moisture level contributed a range of 13 - 25 % in aflatoxin concentration. The degree of detoxification (percentage (%) reduction in aflatoxin) was studied by using 0.1 %, 0.2 %, and 0.3 % aqueous solutions of citric acid. Reduction in aflatoxin %age ranged from 96 % to 99 % was obtained for different curry powder samples by using 0.3 % aqueous solution of citric acid, whereas a 0.2 % aqueous solution of citric acid also yielded good outcomes. This % age reduction in aflatoxin level in acidic medium showed a direct relation with the concentration of citric acid (as concentration was increased, percentage (%) reduction in aflatoxin level increased). Based on the pertinent information regarding the validated method, it can be inferred that this approach has the potential to serve as an effective, economically viable, and time-efficient technique for analyzing and reducing aflatoxins in curry powder and its constituent components.*

**Keywords:** *Aflatoxin, Acetonitrile, Chili, Curry powder, Fungi, Physicochemical*

## INTRODUCTION

Aflatoxins refer to a group of around 20 fungal metabolites that are closely associated with each other. These metabolites are primarily produced by the fungus *Aspergillus parasiticus* and *Aspergillus flavus*, and occasionally by *Aspergillus numinous* (1). The four primary naturally occurring aflatoxins are commonly referred to as $B_1$, $B_2$, $G_1$, and $G_2$. The letters "B" and "G" correspond to the blue and green fluorescent colors observed when

exposed to ultraviolet (UV) light on thin-layer chromatography plates. The subscript numbers 1 and 2 indicate the presence of main and minor chemicals, respectively. Aflatoxin $B_1$ ($AFB_1$), being the most potent of the aflatoxins, is recognized as the most efficacious naturally occurring hepatocarcinogenic chemical (2). Aflatoxin is metabolized by certain P450 enzymes found in the liver, resulting in the formation of a reactive oxygen species known as aflatoxin-8, 9-epoxide (3). This metabolite has the potential to attach to proteins, leading to the manifestation of toxic effects known as aflatoxicosis. Alternatively, it can also bind to DNA, so contributing to the development of liver cancer (4). Aflatoxins (AFs) are considered to be harmful and potent carcinogens, with $AFB_1$ being recognized as the most potent naturally occurring carcinogen for both animals and humans (5, 6). The most commonly seen forms of AFs in food are $B_1$, $B_2$, $G_1$, and $G_2$, collectively representing the entirety of AFs. The presence of AFs is associated with both toxicity and carcinogenicity in populations of both human and animals (4, 6-8). The assessment of aflatoxin (AF) revelation has seen substantial advancements in the past twenty years, mostly driven by the identification and analysis of biomarkers associated with both exposures to aflatoxin and its impact on living organisms. Before quantifying these biomarkers, the predominant method employed to assess human aflatoxin exposure involved observing the average consumption of maize and groundnuts by individuals within specific regions (9). Additionally, estimations were made regarding the aflatoxin content present in these food sources across various global locations (10). Based on the above data, it is possible to derive an approximation for the daily exposure to aflatoxin. Alternatively, the utilization of biomarkers, such as aflatoxin-albumin adducts in serum or aflatoxin-N7-guanine in urine, offers a more accurate means of estimating the levels of aflatoxin present in individuals' diets and the extent to which it has undergone biotransformation, hence increasing the risk of cancer (4). Reduction or elimination of the harmful effects of AF is known as detoxification. Different detoxification methods are reported in literature (11, 12).

Chilies have emerged as a significant commodity in the global spice trade, accounting for approximately 16 % of the market share. This article aims to provide light on the growing prominence of chilies as a trade commodity, particularly in relation to their position in the spice trade following black pepper. The cultivation of *Capsicum frutescence* and *Capsicum annum* is highly valued in Pakistan, as it yields a significant monetary harvest. The allocation of chili production within the nation's gross domestic product (GDP) amounts to 1.5 %. Reports show that Pakistan has been identified as the sixth largest global supplier of chili peppers (13). Contrarily, supplementary data suggests that India serves as the primary exporter of the aforementioned crop, while the quantities of exportation by other countries remain undisclosed. The countries included in the dataset were Spain (17 %), China (24 %), Mexico (8 %), Turkey (4.5 %), Morocco (7 %), and Pakistan (7.25 %). Pakistan ranked fifth in terms of chili production. In Pakistan, chili cultivation spanned across 473,000 hectares, resulting in an output of 70,000 metric tons and a yield of 1.5 metric tons per hectare till seven years prior to the present. The Sindh province accounted for 82 % of the total production, while Punjab provided 10.6 % and Balochistan contributed 6.1 %.

In many countries, chilies are extensively used for flavor and colourant in food where they are usually sun dried by spreading them on ground that provides adulteration with fungi. This poor handling may provide medium for the growth of aflatoxigenic mold (14). On the other hand these dried chilies are usually not stored properly; consequently, chances of mycotoxin producing fungi increases, especially during humid weather (15). Different detoxification technologies like physical, chemical/photochemical, or biological methods are reported to eliminate or inactivate the AFs effects (14, 16) . A study on the different varieties of chilies available in different regions of Lahore, Pakistan, detected the presence of $AFB_1$ in 9 out of 20 samples and they also reported that 2 samples out of the detected 9 samples, ranging between 11.88 ppb (μg $kg^{-1}$) to 11.89 ppb (μg $kg^{-1}$), were beyond the allowed limits (15).

According to ALBORES, the initial step in the detoxification process of $AFB_1$ involves the synthesis of the ß-keto acid structure. This reaction is catalyzed in an acidic environment, and is subsequently followed by the hydrolysis of the lactone ring, resulting in the production of $AFD_1$ (2). The degraded product exhibits similarities to phenolic compounds and is characterized by the absence of lactones derived from the decarboxylation of the lactone ring-opened form of $AFB_1$. While it retains the difurane moiety, it lacks both the lactone carbonyl and the

cyclo pentone ring, which are distinctive features of the $AFB_1$ molecule. Fig. 1 shows the post treatment mechanism of aqueous citric acid on the detoxification of aflatoxin.

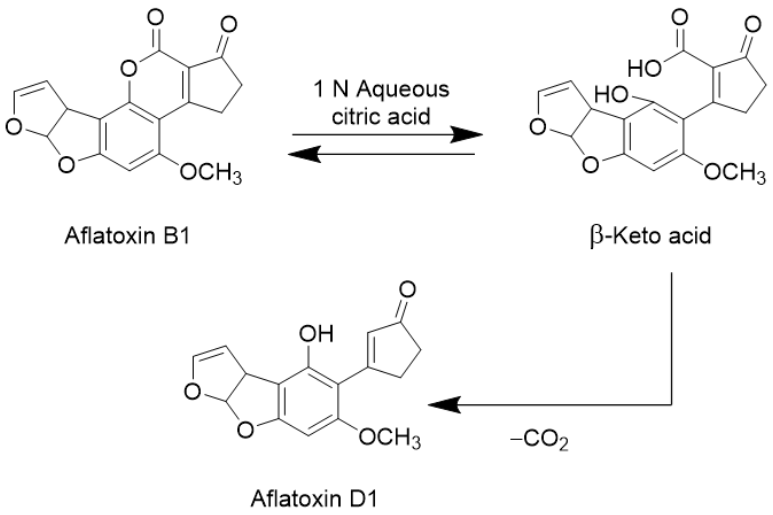


**Fig. 1.** Post-treatment mechanism of aqueous citric acid on the detoxification process of aflatoxin

Though researchers are trying to explore more about AFs and its detoxification, however, in Pakistan determination and detoxification of AFs present in the chilies and curry powder of Faisalabad, Jhelum and Jhung have not been studied yet. Therefore, present study is focused on the determination of mean concentration values of AFs in different curry powder and chili powder samples locally used in Faisalabad, Jhelum and Jhung, as no research work related to these areas has been reported yet. As humidity provides the favorable environment for the growth of moulds, therefore, effects of two different levels of moisture (15 % and 20 %) were also studied to assess the increase in the aflatoxin concentration. Similarly, as acidic medium detoxifies the AFs level, therefore, different concentrations (0.1 %, 0.2 % and 0.3 %) of citric acid were used to find out the detoxification level in chilies powder.

# MATERIALS AND METHODS

## CURRY POWDER SAMPLING

Curry powder is a composite blend of many spices, encompassing coriander (*Coriandrum stivum*), cumin seed (*Cuminum cyminum*), methi (*Trigonella foenum-graecum*), black pepper (*Pipermigrum*), turmeric (*Curcuma longa*), and red pepper (*Capsicum annum*).

## PRETREATMENT OF SAMPLES

The curry powder and its constituent elements were securely maintained within the Food Toxicology Laboratory, located at the Nuclear Institute for Agriculture and Biology (NIAB) in Faisalabad, Pakistan, in order to facilitate the study of aflatoxin levels. Prior to analysis, all samples were ground and in order to inhibit the microbial growth and enzymatic activities, these samples were stored at a moderate freezing temperature of -4°C.

## REAGENTS USED

All chemicals and reagents utilised in the study were of high-performance liquid chromatography (HPLC) grade, including acetonitrile, methanol, n-hexane, glacial acetic acid, trifluoroacetic acid (TFA), and double distilled water. All these reagents were purchased from Sigma Aldrich.

## INSTRUMENTS USED

The available laboratory equipments utilized in present study include: a grinding mill (Retsch ZM 200 model), an analytical balance (Sartorious-1700 model made in Germany), a horizontal shaker, manufactured by Gunter in Germany, nitrogen generator of ANG-238 model-IHC Claind (made in Italy), a vortex mixer/mini shaker

from Thermolyne Sybron in USA, an HPLC system (Shimadzu LC-10A model from Japan), and a fluorescent detector RF-530 model (Shimadzu, made in Japan).

## EXTRACTION EFFICIENCY

The validation experiment adhered to the "Guidance for Industry Bioanalytical Method Validation" as prescribed by the United States Food and Drug Administration (FDA, 2002). Various solvents, including methanol and acetonitrile, either alone or in conjunction with water, were employed in the extraction process of aflatoxins (15) from curry powder samples. Sodium chloride was also considered as an optional component in some of the extraction methods (17). As reported in literature, samples are artificially spiked to find out the efficacy and validity of the AFs analysis (15, 18). In present research, extraction and purification of samples were carried out by using slight modification in the Iqbal et al. methodology (19) and samples were artificially enriched with equivalent quantities of 20 μg $kg^{-1}$ for $AFB_1$ and $AFG_1$, and 10 μg $kg^{-1}$ for $AFB_2$ and $AFG_2$, respectively. Similarly, recovery rates of these AFs during extraction process quantify the extraction process. For extraction and purification, 25 g of fine grinded sample was taken into an acid washed Pyrex glass flask and extracted the aflatoxin with 100 mL acetonitrile: water (84 : 16; v/v) by shaking for 60 minutes at 60 rpm. The extract was filtered through filter paper and 9 mL of the filtrate was poured in a test tube then 70 μL of glacial acetic acid was added in the extract and vortex it for 30 second. This mixture was passed through the Mycosep-226 AflaZon+ column, at a flow rate of 1.5 mL/min and 30 °C column temperature, to filter the contaminants except aflatoxin. Now, 2 mL of elute was evaporated to dryness under a flow of nitrogen at room temperature. The dry residues were dissolved in 200 μL of n-hexane. Later, TFA (50 μL) was added and the solution was vortexed for 30 second and then incubated for 5 min at room temperature for derivatization. A volume of 1.95 mL of Water: ACN (9:1; v/v) was added in to this mixture and vortexed again for 30 second. The derivatized sample was left for 10 min in order to let the layers to be separated. The upper n-hexane layer was discarded. The derivatized sample (50 μL) was injected onto HPLC. Samples were protected from direct light during all procedures.

## EFFECT OF MOISTURE CONTENT ON THE EXTENT OF AFLATOXIN

Moisture levels beyond 13 % and temperatures exceeding 25 °C create an environment that is favorable for the growth and propagation of *Aspergillus* species, resulting in the production of aflatoxin. Aflatoxin contamination has the potential to manifest in capsicum fruits at many times, encompassing pre-harvest, post-harvest, as well as storage and transportation phases. In Pakistan, the process of sun drying red chili often spans a duration of 3 to 7 days, or until the material reaches around 33 % of its original mass. This extended duration allows for the potential accumulation of moisture during some stages, hence creating favorable conditions for the proliferation of aflatoxigenic fungi. The process of drying entails the dispersion of pods in a single layer upon the surface of the soil. The process of sorting is occasionally employed to exclude chilies that have been damaged or affected by illness, however this method is considered basic or elementary. Prior research conducted on various food substrates has demonstrated that the level of mould deterioration is primarily determined by the availability of water (20). In order to conduct the investigation, varying moisture levels (15 % and 20 %) were employed to assess the magnitude of aflatoxin presence. For this purpose, samples were sprayed with distilled water to adjust moisture levels at 15 % and 20 %. For the determination of moisture content level, 5 g of the sample was taken in petri dish and kept in oven at 105 °C for 4 hours. Later, sample was weighed again and the moisture percentage (%) was calculated by using following formula;

$$Moisture\ (\%) = \frac{\text{weight of sample after moisture loss (g)}}{Original\ weight\ of\ sample\ (g)}\ X\ 100$$

Samples having 15 % and 20 % moisture were kept at 40 °C for 1 hour and then kept in incubator at 28 °C for 7 days for the determination of level of AFs in these samples.

## DETOXIFICATION OF AFLATOXINS

While there have been established therapies aimed at reducing the levels of certain mycotoxins, no single approach has been found that is equally effective against the diverse range of mycotoxins that may coexist in different food commodities. One often employed approach for mitigating the presence of mycotoxins is lowering

their bioavailability through the incorporation of diverse mycotoxin binding agents or adsorbents. Irradiation has demonstrated efficacy in reducing aflatoxins (AFs) in food products, while certain compounds such as citric acid, propionic acid, and synthetic antioxidants have also been found to inhibit fungal growth and decrease AF levels in laboratory settings. In the conducted investigation, the utilization of citric acid has been employed as a mean to reduce fungal and AFs contamination in curry powder and its constituent ingredients with slight modifications in the reported methods (21-23). For this purpose, samples with high intensity of aflatoxins were selected and different concentrations (0.1 %, 0.2 % and 0.3 %) of citric acid were exposed to samples at 1 mL/gm for a contact time of 24 hours at room temperature.

## STATISTICAL ANALYSIS

The laboratory sample was partitioned into three analytical samples, and subsequently, each sample underwent triplicate analysis. The resulting data are shown as the mean value (n = 3 x 3) ± SD (n = 3 x 3). The statistical programme used for conducting the analysis of variance (ANOVA) was Minitab 2000 version 13.2, developed by Minitab Inc. in Pennsylvania, USA. Statistical analysis was conducted to determine significant differences ($p < 0.05$) in the mean values, employing Duncan's multiple range tests. Statistical significance was determined based on a threshold of $p \leq 0.05$.

# RESULTS

Based on the aforementioned statistics, it has been observed that the recovery percentage of aflatoxins in the constituents of curry powder is equal to or more than 88 %. The presence of the co-extract in the sample did not have an impact on the recovery of aflatoxins. The solvent employed, consisting of a mixture of acetonitrile and water, exhibited favorable characteristics in effectively capturing the hydrophobic aflatoxins present in all spice components. Table I shows the recovery % of aflatoxins in curry powder, chili and coriander. Table II shows the parameters of linear regression whereas, Fig. 2 (a-d), shows the linear behavior of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$, respectively. Fig. 3 shows the resolution of standard aflatoxin mixture solution by using HPLC with Flow rate: 1.5 mL $min^{-1}$, Column temp: 30°C; Detector: FLD, wavelength: EX: 360 nm; EM: 440 nm, Column: $C_{18}$ (Discovery); 250 x 4.6 mm, 5 µm, Injection volume: 20 µL, Pressure: 184 $kgf/cm^2$.

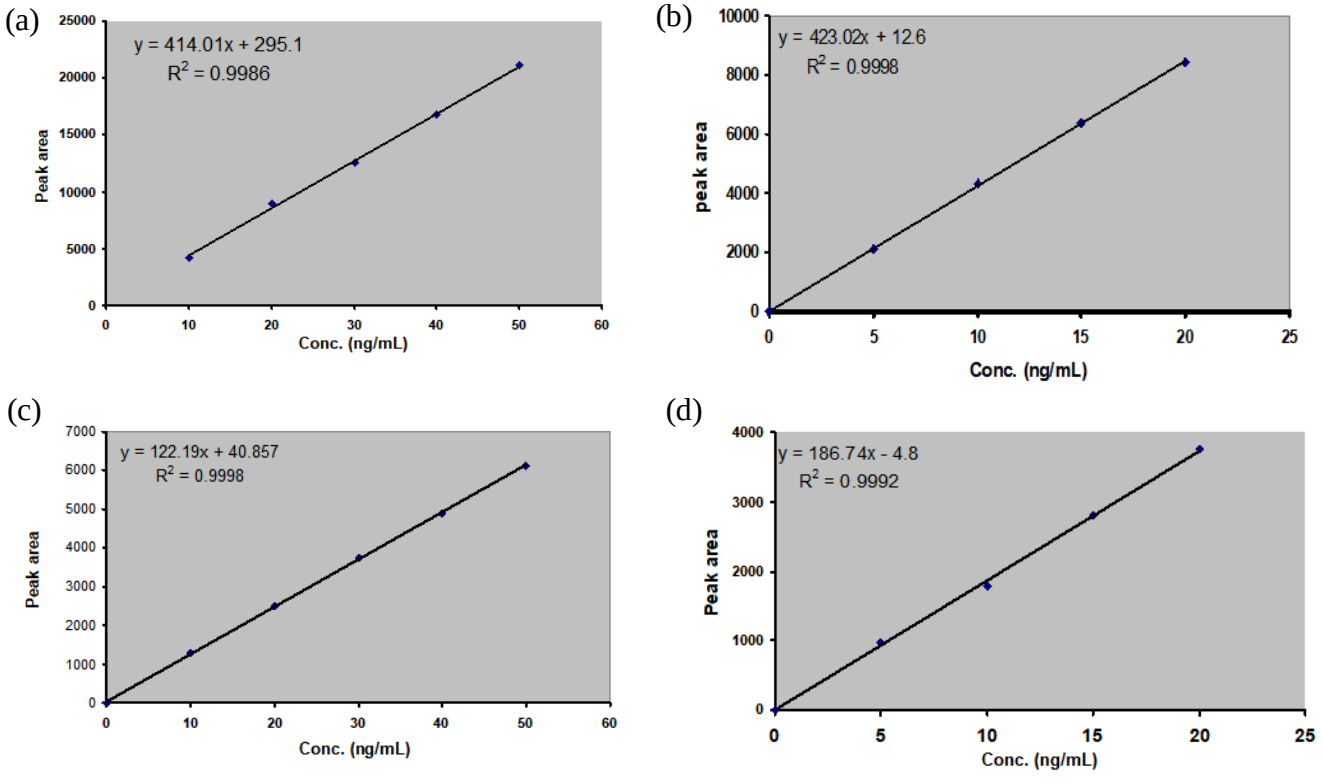


**Fig. 2 (a).** Linear behavior of $AFB_1$ determined from chromatogram of HPLC data; **(b).** Linear behaviour of $AFB_2$ determined from chromatogram of HPLC data; **(c).** Linear behaviour of $AFG_1$ determined from chromatogram of HPLC data; **(d).** Linear behaviour of $AFG_2$ determined from chromatogram of HPLC data

**Table I.** Recovery Percentage of aflatoxins in chilies, coriander and cumin seed

| Aflatoxin | Spiking level (µg kg$^{-1}$) | Chilies | Coriander | Cumin eed | Over all Mean ± S.D |
|---|---|---|---|---|---|
| $AFB_1$ | 20 | 2 | 6 | 90 | 89 ± 3.05 |
| $AFB_2$ | 10 | 90 | 8 | 94 | 1 ± 3.05 |
| $AFG_1$ | 20 | 94 | 0 | 6 | 90 ± 4.00 |
| $AFG_2$ | 10 | 4 | 86 | 84 | 8 ± 5.29 |

Data is mean of 3 replicates ± standard deviation

**Table II.** Parameters of linear regression* measured for aflatoxins ($AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$) in HPLC

| Aflatoxin | Concentration | Slope (a) | Intercept (b) | $R^2$ |
|---|---|---|---|---|
| $AFB_1$ | 10-50 ng mL$^{-1}$ | 295.10 | 414.01 | 9986 |
| $AFB_2$ | 5-20 ng mL$^{-1}$ | 1206 | 423.02 | 9998 |
| $AFG_1$ | 10-50 ng mL$^{-1}$ | 40.86 | 122.19 | 9998 |
| $AFG_2$ | 5-20 ng mL$^{-1}$ | 04.80 | 186.74 | 9992 |

*y = ax + b; y = peak area, x = ng injected, $R^2$ = regression coefficient

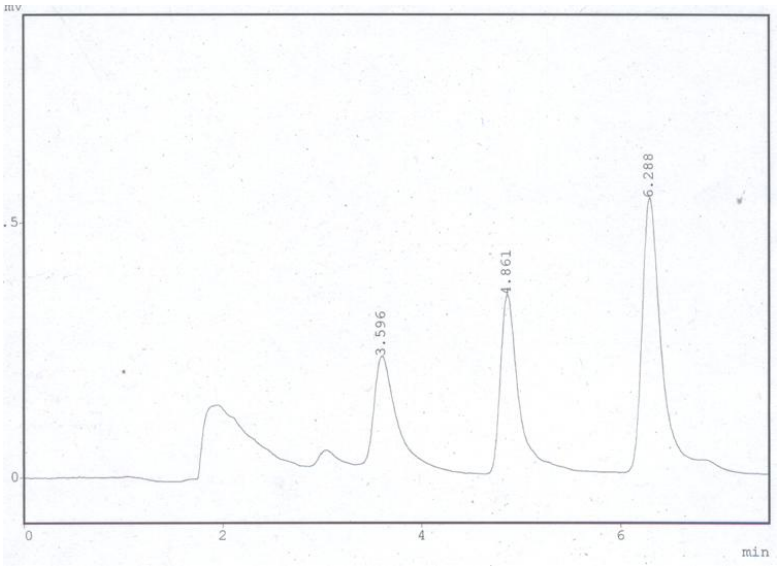


**Fig. 3.** Chromatogram, between time retention in minutes along x-axis and detector response in mV along y-axis, showing resolution of standard aflatoxin mixture solution by HPLC

## OCCURRENCE OF AFLATOXINS IN CHILIES SAMPLES

The analysis of various samples of curry powder and its constituent ingredients revealed that a significant majority of the samples, specifically 88 %, were found to be contaminated with diverse forms of aflatoxins. It was determined through verification that among a total of 40 chili samples obtained from Faisalabad, 36 (90 %) exhibited contamination with an average aflatoxin concentration of 65 ± 5.79 µg kg$^{-1}$. In comparison, the contamination rates in samples taken from Jehlum and Jhang were found to be 90 % and 80 % respectively. The samples collected from Jehlum had the highest overall mean value of aflatoxins i.e. 69.5 ± 6.44 µg kg$^{-1}$. Table III shows the aflatoxin level and Table IV shows the distribution of aflatoxin levels in chilies collected from Faisalabad, Jehlum and Jhang. Graph between mean levels of aflatoxins versus number of contaminated samples is shown in Fig. 4.

**Table III.** Aflatoxins* level (µg kg$^{-1}$) in Chilies samples collected from Faisalabad, Jehlum and Jhang areas

| Commodity | Area | Total samples | Contaminated samples (%) | Mean ± SD (µg kg$^{-1}$) |
|---|---|---|---|---|
| Chilies | Faisalabad | 40 | 36 (90) | 65 ± 5.79 |
| Chilies | Jehlum | 20 | 18 (90) | 69.5 ± 6.44 |
| Chilies | Jhang | 15 | 12 (80) | 31 ± 10.02 |

*Aflatoxins = $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$

**Table IV.** Distribution of aflatoxin levels in chilies collected from Faisalabad, Jehlum and Jhang

| Area of samples | Total samples | Contaminated samples (%) | Chilies aflatoxin > 20 μg kg$^{-1}$ (%) | | |
|---|---|---|---|---|---|
| | | | 21 - 25 μg kg$^{-1}$ | 26 - 30 μg kg$^{-1}$ | 30 μg kg$^{-1}$ |
| Faisalabad | 40 | 36 (90) | Nil | 4 (10) | 32 (80) |
| Jehlum | 20 | 18 (90) | 2 (10) | 2 (10) | 14 (70) |
| Jhang | 15 | 12 (80) | Nil | 4 (27) | 8 (53) |
| Total | 75 | 66 (88) | 2 (3) | 10 (13) | 54 (72) |

Acceptable upper limit for aflatoxin in grains is 20 (μg kg$^{-1}$) (FDA, 2002)

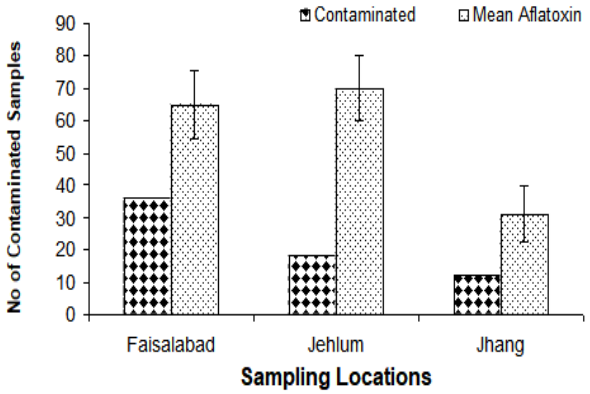


**Fig. 4.** Contaminated (%) and mean level of aflatoxins in chili samples collected from different cities of Punjab, Pakistan

**Table V.** Occurrence of the four major types of aflatoxins in Chilies

| Aflatoxin | No. of sample | Percentage (%) | Conc. Range (μg kg$^{-1}$) | Sample |
|---|---|---|---|---|
| $AFB_1$ | 70 | 93 | 0.5 - 65 | Chilies |
| $AFB_1 + AFB_2$ | 20 | 26 | 6 - 38 | Chilies |
| $AFG_1 + AFG_2$ | 16 | 21 | 0.2 - 6 | Chilies |
| Total aflatoxin | 12 | 16 | 6 - 62 | Chilies |

*samples were analyzed with Fluorescence detector and compared the concentration with working standard

Table V shows the occurrence of four major types of aflatoxins in chilies and the graphical representation in Figure 5 illustrates the percentage distribution of chilies throughout several regions within the Faisalabad Division. The incidence of $AFB_1$ in chili peppers exhibited a significantly greater prevalence in comparison to the overall occurrence of aflatoxins ($AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$).

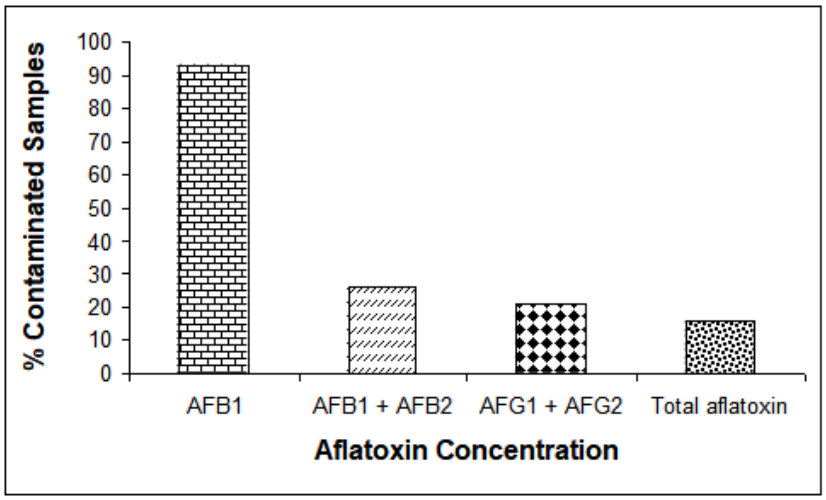


**Fig. 5.** Distribution percentage of different aflatoxins in chili samples

The coexistence of fungi does not inherently indicate the existence of poisons. Fungal species have the capacity to generate aflatoxins on agricultural commodities either during periods of stress in the field or during

storage, particularly when exposed to conditions characterized by high moisture and warm temperatures ranging from 25 to 30 °C. Global surveillance programs for aflatoxins in different agricultural commodities have been recommended and recently updated and acknowledged due to the potential risks they pose to human and animal health. Table VI shows the aflatoxins level (µg $kg^{-1}$) in Curry powder samples and Fig. 6 shows the graph between contaminated samples and average value of aflatoxin in curry powder.

**Table VI.** Aflatoxins* level (µg $kg^{-1}$) in Curry powder samples collected from Faisalabad, Jehlum and Jhang

| Commodity | Area | Total samples | Contaminated samples (%) | *Mean ± SD (µg $kg^{-1}$) |
|---|---|---|---|---|
| Curry Powder | Faisalabad | 40 | 35 (88) | 35 ± 10.4 |
| Curry Powder | Jehlum | 20 | 14 (70) | 39 ± 10.07 |
| Curry Powder | Jhang | 15 | 11 (73) | 27 ± 8.82 |

*Aflatoxins = $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$

Certain emerging countries, including as China and Mexico, have implemented restrictions that align with those of the United States in terms of human consumption and trading. Due to the implementation of food safety rules, cereals containing elevated levels of aflatoxins are forbidden from both domestic and international trade.

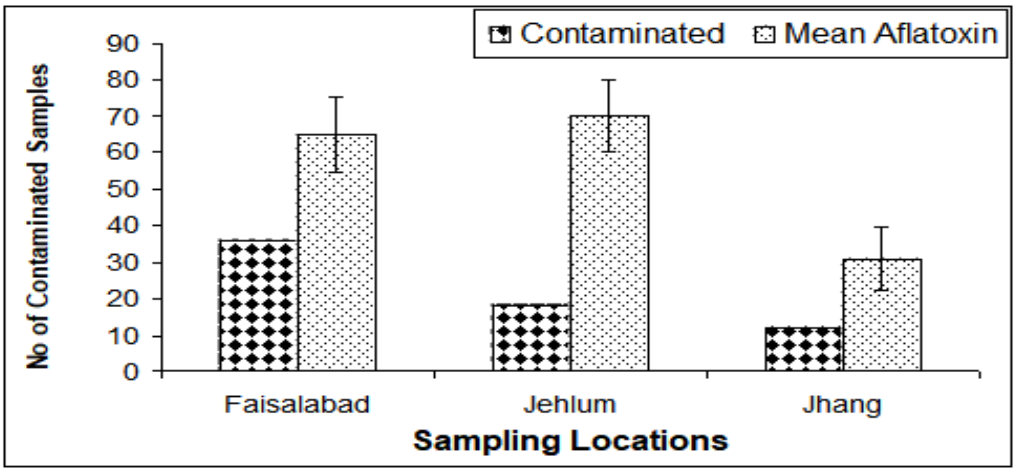


**Fig. 6.** Contaminated sample and mean (average) value of aflatoxins in curry powder taken from different cities of Punjab, Pakistan

The consumption of curry powder by residents residing in Faisalabad, Jehlum, and Jhang may lead to the development of certain ailments as a result of the harmful nature of aflatoxins. Table VII shows the occurrence of the four major types of aflatoxins in Curry powder from different areas. The concentration of aflatoxins was determined by calculating the peak area for each component. The data presented in Table VIII were juxtaposed with the prescribed aflatoxin thresholds for different food items in various nations, as depicted in Table VI. The findings of the current study reveal that out of the 75 samples analyzed, 69 samples were found to possess residues of $AFB_1$ ranging from 9 to 66 µg $kg^{-1}$, while 22 samples included residues of both $AFB_1$ and $AFB_2$ ranging from 12 to 40 µg $kg^{-1}$. In the study conducted, it was shown that 11 % of the examined samples contained aflatoxins (specifically $AFG_1$+$AFG_2$) with concentrations ranging from 2 - 25 µg $kg^{-1}$. Additionally, a smaller percentage of samples (19 %) exhibited residues of total aflatoxin within the range of 20 - 64 µg $kg^{-1}$. Fig. 7 depicts the graph between sample contamination and percentage (%) incidence of aflatoxin whereas Fig. 8 shows the sample contamination and percentage (%) incidence of aflatoxin in ingredients of curry powder.

**Table VII.** Occurrence of the four major types of aflatoxins in Curry powder from different areas

| Aflatoxin | No. of sample | ercentage (%) | Conc. Range (µg $kg^{-1}$) | Condition of sample |
|---|---|---|---|---|
| $AFB_1$ | 69 | 92 | 9 - 66 | Curry Powder |
| $AFB_1$ + $AFB_2$ | 22 | 29 | 12 - 40 | Curry Powder |
| $AFG_1$ + $AFG_2$ | 8 | 11 | 2 - 25 | Curry Powder |
| **Total aflatoxin** | **14** | **19** | **20 - 64** | **Curry Powder** |

*samples were analyzed with Fluorescence detector and compared the concentration with working standard

**Table VIII.** Aflatoxins* level (µg $kg^{-1}$) in ingredients of curry powder samples collected from Faisalabad, Jehlum and Jhang

| Commodity | Area | Total samples | Contaminated samples (%) | *Mean ± SD (µg $kg^{-1}$) |
|---|---|---|---|---|
| Coriander | Faisalabad | 15 | 4 (27) | 7 ± 0.98 |
| Cumin seed | Jehlum | 15 | 5 (33) | 6 ± 0.58 |
| Methi | Jhang | 15 | 3 (20) | 2.5 ± 0.30 |
| Black pepper | Faisalabad | 10 | 4 (40) | 4 ± 0.52 |
| Metha | Faisalabad | 15 | 5 (33) | 1.5 ± 0.54 |
| Turmeric | Jehlum | 15 | 4 (27) | 3.5 ± 0.43 |

*Aflatoxins = $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$

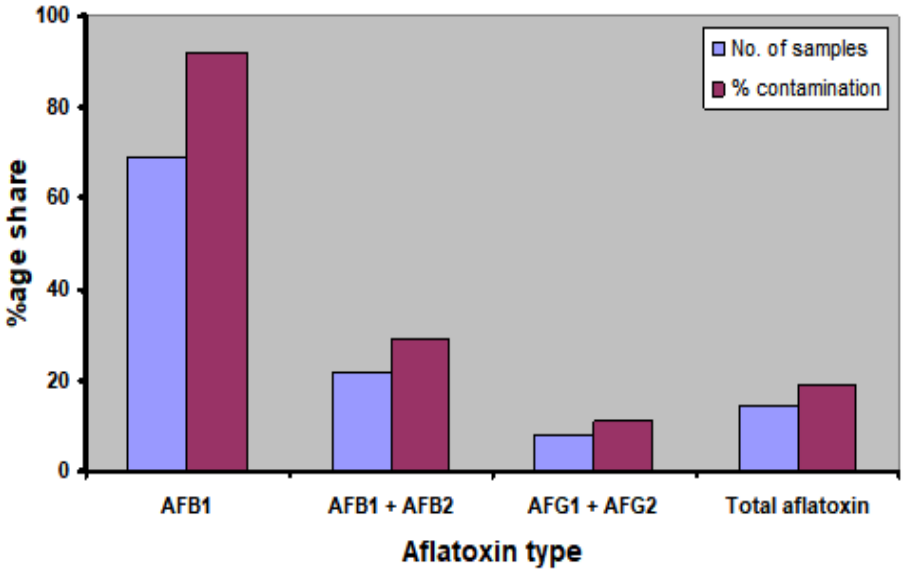


**Fig. 7.** Samples contamination and percentage (%) incidence of aflatoxins in Curry Powder

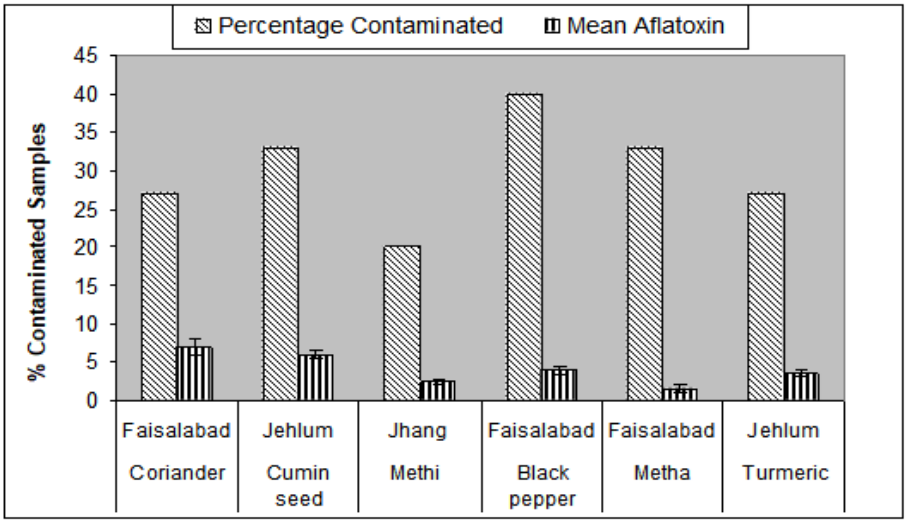


**Fig. 8.** Samples contamination and percentage (%) incidence of aflatoxins in ingredients of Curry Powder

**Table IX.** Effect of moisture content (%) on the production of aflatoxin on exposure of environment for specific time

| Nature of sample | Initial level of aflatoxin (µg $kg^{-1}$) | Moisture level (%) | | Duration of exposure to moisture |
|---|---|---|---|---|
| | | 15 | 20 | |
| Red chili powder-Faisalabad | 25 | 27 | 28.5 | weekly |
| Red chili powder-Jehlum | 30 | 34 | 36 | weekly |
| Curry powder-Faisalabad | 20 | 25 | 27 | weekly |
| Curry powder-Jehlum | 40 | 45 | 47 | weekly |

The findings (as shown in Table IX) clearly indicates that moisture levels have a key role in the development of aflatoxin in red chili powder and curry powder. The presence of moisture at a level of 15 % was shown to result in an elevation of aflatoxin levels in red chili powder, ranging from 8 % to 13 %. Similarly, in curry powder, the observed increase in aflatoxin levels ranged from 13 % to 25 %. The findings of the experiment conducted under a moisture level of 20 % demonstrated similar trends in the production of aflatoxins. Nevertheless, a significant modification was noted in the curry powder acquired from the Faisalabad market, exhibiting a moisture content of 35 %. This study investigates the potential of citric acid to mitigate aflatoxins in curry powder. Among the different forms of aflatoxin toxicity, the aspect of carcinogenicity has garnered significant interest, prompting numerous investigations aimed at identifying chemoprotective drugs capable of mitigating aflatoxin-induced carcinogenic effects. Various methodologies have been employed in the detoxification of aflatoxins; yet, only a limited number of these methodologies have demonstrated practical utility. One of the methods that have been found to be successful and cost-efficient in lowering the aflatoxin level of various foods is ammoniation. Citric acid solutions (Merck, Germany) in aqueous form were employed for the purpose of aflatoxin reduction in curry powder.

**Table X.** Effect of citric acid on the detoxification of aflatoxin in curry powder using different concentrations of citric acid showing percentage (%) reduction in aflatoxin

| Nature of sample | Level of aflatoxin ($\mu g\ kg^{-1}$) | Concentration of aqueous citric acid (%) | | |
|---|---|---|---|---|
| | | 0.1 | 0.2 | 0.3 |
| Curry powder | 46 | 30 (35%) | 10 (78%) | 2.0 (96%) |
| Curry powder | 35 | 20 (43%) | 8 (77%) | 1.5 (96%) |
| Curry powder | 24 | 16 (33%) | 6 (75%) | 0.2 (99%) |

*Triplicate samples were used for this study. Values in parenthesis showed reduction percentage

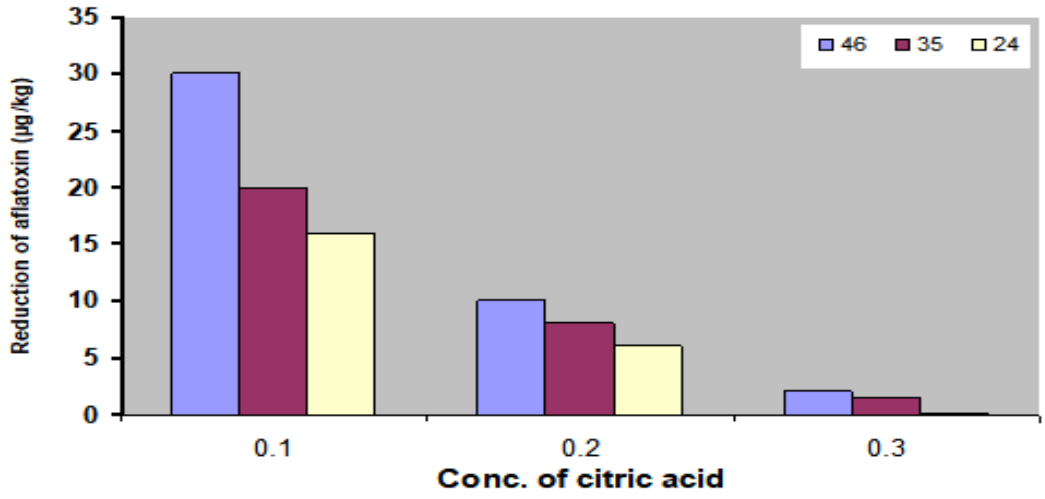


**Fig. 9.** Effect of various concentrations of aqueous citric acid (percentage (%) solutions) on the detoxification of aflatoxin

The study involved the selection of curry powder samples exhibiting significant levels of aflatoxins, which were subsequently subjected to treatment using varying doses of citric acid as shown in Table X. The application of various concentrations of citric acid resulted in a considerable reduction in aflatoxin levels in curry powder. Notably, the concentration of 0.3 % exhibited the highest efficacy among the tested concentrations. The level of detoxification ranged from 96 % to 99 % when using a 0.3 % aqueous solution of citric acid, whereas a 0.2 % aqueous solution of citric acid yielded good outcomes. Fig. 9 highlights the effect of aqueous citric acid on the detoxification of aflatoxin.

# DISCUSSION

Mycotoxins are a distinct category of small-sized chemicals that are found in various dietary sources and have an impact on animal organisms, including humans (2). The production of these substances is attributed to

filamentous fungi, however the presence of the fungi in the food may no longer be detectable. It is imperative to acknowledge that mycotoxins do not possess optimal efficacy as weapons. For instance, there may exist other fungal toxins that are not present in food and possess higher levels of toxicity. Therefore, possessing a comprehensive understanding of the correlation between fungus and their corresponding toxins, commonly referred to as fungal chemotaxonomy, is of utmost importance. Mycotoxins refer to hazardous secondary metabolites generated by fungi, generally known as moulds, which inhabit agricultural crops during cultivation or after harvesting. Consequently, these mycotoxins present a potential risk to the well-being of both human and animals (24). Certain types of moulds have the ability to create mycotoxins, which are commonly known as toxigenic moulds. The primary fungal genera responsible for the production of mycotoxins include *Aspergillus*, *Fusarium*, and *Penicillium* (25). Numerous fungal species have the capacity to generate mycotoxins. The growth of moulds and subsequent mycotoxin production can occur at various stages, including pre-harvest, during transportation, processing, or storage.

The approach outlined in the specified section was employed to extract aflatoxins ($B_1$, $B_2$, $G_1$, and $G_2$) from the samples. The majority of authors employed immunoaffinity columns for the purpose of purifying the extract, and subsequently conducted high-performance liquid chromatography (HPLC) measurements in accordance with the contained instructions for the use of immunoaffinity columns (26). In the conducted investigations, Mycosep-226 clean-up columns were utilized, resulting in a high recovery rate of the spiked concentration and the absence of any interfering compounds (peaks) in the chromatograms.

The contamination percentage in Jehlum was found to be higher in comparison to that of Faisalabad and Jhang. Based on the gathered data, it is evident that a majority of residents opt to store chilies for extended duration in fabric bags, exposing them directly to open air and sunlight, which has a notable impact on the chili storage process. The potential correlation between elevated temperatures and the presence of aflatoxins is attributed to the favorable conditions that promote the rapid growth and proliferation of toxigenic fungi. The aforementioned rationale was also cited by the researcher who deduced that the synthesis of AFs and the proliferation of the fungi responsible for their creation are contingent upon various parameters, including temperature, humidity, handling practices during harvesting, and storage conditions.

The chili peppers and other constituent elements of curry powder cultivated in Faisalabad, Jehlum, and Jhang are disseminated to various regions of Pakistan, with a particular focus on the southern and northern areas. These regions experience agricultural challenges stemming from their geographical characteristics and climatic conditions.

The mean aflatoxins in collected samples were 20 – 91 μg $kg^{-1}$ whereas overall 72 % samples of chilies contained ≥ 30 μg $kg^{-1}$. Additional factors contribute to the presence of aflatoxin contamination in chili samples, which were not previously seen in research investigations. A study was conducted to investigate the impact of moisture on the levels of aflatoxins in curry powder and chili powder (27). The outcomes of the samples subsequent to the application of moisture at levels of 15 % and 20 % are documented in the Table IX. The aflatoxin concentration exhibited an increase in samples characterized by elevated moisture levels. The current findings are compared with the significance of moisture levels in relation to the presence and severity of aflatoxins is apparent.

The present research work has investigated the impact of aqueous citric acid on the process of detoxifying aflatoxins. In this study, various concentrations (0.1 %, 0.2 %, and 0.3 %) of aqueous citric acid were administered to chilies and curry powder. The obtained outcomes were subsequently compared to the results obtained from another experimental condition. In the conducted investigation, it was shown that the utilization of a 0.3 % aqueous solution of citric acid demonstrated enhanced efficacy in the process of detoxifying aflatoxins. Table X indicates that the use of a 0.3 % aqueous citric acid treatment resulted in a reduction of aflatoxin levels in curry powder by 99 %.

## CONCLUSION

The present study employs a validated methodology utilizing the MycoSep-226 technique followed by liquid chromatographic separation with fluorescence detection. This method is utilized for the accurate determination of aflatoxins produced by fungi in curry powder and its constituent ingredients. The evaluation of

various parameters including recovery, precision, limit of detection, limit of quantitation, reproducibility, and repeatability demonstrates results that align with the published data and adhere to the guidelines set forth by Codex. Based on the presented empirical evidence, it is evident that the utilization of a validated methodology can yield significant benefits in terms of efficiency, cost-effectiveness, and time savings when analyzing aflatoxins in curry powder and its constituent ingredients. The significance of the effects of moisture, temperature, storage, and citric acid on the levels of aflatoxins in curry powder and its constituent constituents is well-founded. With the exception of the action of citric acid, the aforementioned factors contribute to a rise in aflatoxin levels. However, the presence of aqueous citric acid has been found to decrease aflatoxin levels in curry powder.

## Conflict of Interest:

Authors have no conflict of interest.

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