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Detection and Detoxification of Aflatoxins in Corn Grain and Corn Flour Collected from Different Areas of Lahore

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Abstract. Aflatoxins are naturally occurring toxic metabolites produced by several fungi. Aflatoxins are responsible for contamination of animal feed and human food which in turn causes the detrimental effects which mainly includes carcinogenicity, stunted growth, hepatocellular carcinoma and liver cirrhosis. Amongst the aflatoxins, AFB_1 is designated as a potent hepatocarcinogen in humans. This study is aimed to detect and detoxify different types of aflatoxins in corn grain collected from Lahore and Kasur, Pakistan. Overall samples (n=80) were collected for the determination of aflatoxins by thin layer chromatography (TLC). The samples collected from Lahore (n=40), the AFB_1 was detected in 12 samples (30%) samples, and similarly from Kasur AFB_1 was detected in 9 samples (22%). The concentration of AFB_1 in samples ranged from 1.51 to 38.54 µg/Kg. The second phase of the study was focussed on the use of different detoxification methods. Various detoxification strategies were used to degrade AFB_1 in contaminated corn *i.e*. physical, chemical and biological method. Maximum reduction in AFB_1 level was obtained by using 20% citric acid, which reduced 38.54 µg/Kg to 18.23 µg/Kg (52.6%), mustard oil reduced 38.08 µg/Kg to 18.22 µg/Kg (52.1%) and boiling method reduced 38.54 µg/Kg to 20.91 (45.7%) in corn samples. It can be concluded from the current study that corn is highly susceptible to fungal contamination and produces AFB_1 beyond permissible limits. The improvement in storage conditions and use of detoxification methods can help us reduce the fungal contamination and avoid health related risks due to presence of aflatoxins.

Keywords: aflatoxins, detection, corn, detoxification, thin layer chromatography, AFB_1

Introduction

Aflatoxins are considered as one of the most potent naturally produced mycotoxins associated with agricultural products that is hazardous for human and animal health (Kumar *et al*., 2017). Mycotoxins are secondary metabolites produced by moulds or filamentous fungi. *Aspergillus*, *Alternaria*, *Penicillium* and *Fusarium* are the most significant fungal genera producing mycotoxins. Crops are infected by mycotoxic fungi at many phases, including in the field through contaminated soil, during harvesting and post-harvesting (Berthiller *et al*., 2013). Mycotoxins commonly contaminate food or feed especially cereals and have toxicological impact on both human and livestock. Several mycotoxins found in food have long-term effect on human health resulting in development of cancer, mycotoxicosis, ergotism and immunological deficiencies. Mycotoxins are classified into five categories *i.e*. zearalenone, deoxynivalenol, fumonisins, ochratoxin and aflatoxins (Pohland, 1993).

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Aflatoxins are naturally existing secondary metabolites mostly produced by *Aspergillus* sp. *i.e*. *Aspergillus flavus* and *Aspergillus parasiticus* (Nazir *et al*., 2019). *A. flavus* and *A. parasiticus* are well known to produce aflatoxin. Mycotoxins produced by *Aspergillus* species such as *A. flavus, A. parasiticus* and *A. nomius* among others which are referred to as aflatoxins (Kumar *et al*., 2018; Gacem *et al*., 2016; Monson *et al*., 2015). Aflatoxins are majorly classified into three groups *i.e*. $AFB_{(1,2)}$, $AFG_{(1,2)}$, $AFM_{(1,2)}$. Aflatoxin B_1 (AFB_1), followed by aflatoxin G_1 (AFG_1), aflatoxin B_2 (AFB_2) and aflatoxin G_2 (AFG_2), is the highly toxic compound that is typically found in food and feed materials under natural conditions. According to international agency for research on cancer (Heinrich, 2003) AFB_1 and AFM_1 are called as group 1 human carcinogens (Chavez *et al*., 2020). A food crop or product is likely to get infected by AFB_1, AFB_2 and AFG_1, whereas a dairy product is likely to have AFM_1 and AFM_2. Both AFB_1 and AFB_2 may be found in a wide range of foods, including wheat, sorghum, peanuts and soybeans. Other spices, such as

black and chilli peppers, may also be contaminated (Rajarajan *et al*., 2013). Exposure to aflatoxins proved very harmful for humans, animals and livestock. They can also cause irreversible loss to world's economy by contaminating crop produced. Aflatoxins aggravates the situation by damaging world food, leading to more hunger and possibility of famine due to food shortage in different parts of world. As indicated by IARC (International Agency for Research on Cancer (IARC), aflatoxins are menacing for human health and may induce many health complications (Heinrich, 2003). Humans can also get affected directly or indirectly by aflatoxins *i.e*. by consuming contaminated milk or egg (Qureshi *et al*., 2015). Generally, aflatoxin consumption causes abdominal pain, vomiting, nausea, acute convulsions and chronic exposure can result in major complications such as immunotoxicity, hepatotoxicity and teratogenicity. Aflatoxin particularly affects children and associated with delayed development, stunted growth and liver abnormalities. (Liu and Wu, 2010). Aflatoxicosis is a disease caused by aflatoxin consumption and may results in death. Acute aflatoxicosis happens as a result of high-level exposure of aflatoxins and cause bleeding, abnormal digestion, malabsorption, edema, mental dysfunctioning and liver tissue necrosis leading to hepatocellular-carcinoma (Williams *et al*., 2004). Aflatoxin contamination in food and feed occur during and after harvest due to mishandling or improper storage (Benkerroum *et al*., 2019; Rushing and Selim, 2019). Seasonality, post-harvest, management activities, food type and geographic location are the controlling factors for aflatoxin invasion in crops (Khaneghah *et al*., 2018). The presence of light has an impact on fungi growth and aflatoxin generation. Aflatoxin production is increased in the dark, while it is inhibited in the light (Rushing and Selim, 2019). A lower O_2 concentration and a higher CO_2 concentration inhibit an elevated level of aflatoxins (Mahbobinejhad *et al*., 2019). It is impossible to completely degrade aflatoxin from food therefore various countries set permissible limit on aflatoxin level in different food items. U.S. Food and Drug Administration regulations set aflatoxins limits of 20 µg/Kg for food or feed and 0.5% for milk products (Ibrahim and Menkovska, 2018). The European Union also interpolates AFB_1 levels in food commodities in the range of 2-4 µg/Kg. As aflatoxins are hazardous to livestock, it is necessary to remove them from these sources in order to prevent financial harm from aflatoxin contamination of a variety of foods and feeds. In a recent study aflatoxin quantification was performed in maize grains obtained from 12 different districts of Punjab by using high performance liquid chromatography (Manzoor *et al*., 2018). This current study focuses on aflatoxin detection and detoxification in corn grain and corn flour collected from 2 specific districts of Punjab *i.e*. Lahore and Kasur.

Various strategies have been devised for identification of aflatoxins in food products. Chromatography is most commonly used method for the assessment and quantitative evaluation of aflatoxins. Thin layer chromatography (TLC) and High-performance liquid chromatography (HPLC) is widely used for identification of various mycotoxins (Jinap *et al*., 2012). In present study detection of aflatoxin in corn is done by using thin layer chromatography as it is rapid and cost effective method. For de-toxification of aflatoxins three different methods were employed *i.e*. physical, biological and chemical. As maize is the fourth most important crop of Pakistan. Therefore, necessary actions needs to be taken in order to prevent or control aflatoxins invasion in maize crops. The objective of this research is to identify and detoxify aflatoxins from the corn (grain and flour). This study provides recent and current updates regarding aflatoxin contamination of corn in year 2021 in district Lahore and Kasur.

Materials and Methods

Sampling and storage. All samples (n=80) of maize grains and corn flour were collected from various places of Lahore and Kasur in the duration of March-May 2021. Following the sampling the entire samples of corn were stored in polythene bags in a cold, dry place until the analysis.

Aflatoxin extraction and characterization. For aflatoxin analysis, chemical reagents used were: choloroform, diethyl ether, acetone, celite, sulphuric acid and aflatoxin standard solution. For chemical detoxification, 5% Sodium bicarbonate, 20% of citric acid, 10% acetic acid and 0.5% HCL were employed. For aflatoxin extraction following process was done. Initially 50 g of crushed maize grains were placed in a 500 mL conical flask. 25 mL water and 150 mL chloroform were added to a conical flask containing grinded maize grains. The sample solution in the conical flask was stirred for 25-30 min using a wrist motion shaker. The sample solution in the conical flask was filtered using Whatman 4 filter paper. In a beaker, the

filtrate (50 mL of $CHCl_3$) was collected and allowed to evaporate on a hot plate. Aflatoxin identification and assessment was done by comparing it to aflatoxin standard, according to the Association of Official Analytical Chemists (AOAC) 2005.

TLC (thin layer chromatography) is a qualitative method used for detecting aflatoxin in current study. It is a simple and cost-effective method of quantification. It is quite easy to use. UV light is used to detect and identify mycotoxins (Holcomb *et al*., 1992). Sample of 5, 10, 15 and 25 L were observed on a TLC plate. Spots of 5 or 10 µL were put on the same TLC plate as the standards. For TLC plate development place the plate in a chromatographic tank containing diethyl ether and allowed to settle to half its original size. After development in tank-1, plate was removed and dried. The TLC plate was redeveloped in chromatographic tank-2, which was filled with a 9:1 chloroform/acetone mixture. The TLC produced plate was analysed and the presence or absence of aflatoxin was recorded under UV light at a wavelength of 365 nm (Nisa *et al*., 2014). Sample spots were compared to standard spots in terms of fluorescence.

One of the most important methods in aflatoxins analysis is the fluorescing of sample spots. This was performed by uniformly spraying aq. sulphuric acid (50/50 v/v) over the TLC plate. The TLC plate was then dried and examined under a 365 nm Ultra-violet light (Nisa *et al*., 2014).

Calculations. The concentration of aflatoxins (µg/Kg) in the samples was calculated according to the formula:

Aflatoxin concentration (mg/Kg)=S×Y×V/W×Z

where:

S=Volume (µL) of aflatoxins standard of equivalent intensity to Z (µL of sample); Y=Concentration of aflatoxins standard in µg/mL; V=Solvents volume (µL) of solvents needed to dilute final extract; Z=Volume (µL) of sample extract required for spotting; W=Weight (g) of original sample contained in final extract.

Detoxification. In present study decontamination of positive samples was performed in a variety of ways. Samples were detoxified by physical, chemical, biochemical (using plant extracts) methods etc.

Physical method. One severely contaminated sample was chosen from the contaminated samples for physical control detoxification. To destroy aflatoxins in maize, heat can be applied in the form of boiling, roasting, baking, or steaming. Following de-toxification, the level of aflatoxin was revaluated using the same TLC approach as previously indicated.

Chemical method. One of the most contaminated samples from the positive samples was tested for aflatoxin reduction using a chemical treatment. For decreasing contamination ratios in chosen samples, several organic and inorganic acids were utilised. By following the process of Zahra *et al.* (2012), 50 g of ground sample was taken in different conical flasks containing 0.5% hydrochloric acid, 5% sodium bicarbonate, 10% acetic acid and 20% citric acid solution. After being shaken on a wrist action shaker for 2 h, solution in conical flasks were filtered and kept for drying for two days. After de-contamination, AFB_1 quantification was performed by following similar method *i.e*. aflatoxin extraction and characterization and aflatoxin level estimation by formula.

Biological method. AFB_1 in contaminated samples was reduced using biological resources. We used black seed oil, ginger paste, garlic paste and mustard oil. Lh18, one of the most contaminated samples, was chosen for biodegradation for this purpose. The natural detoxification process was carried out using Vijayanandraj *et al.* (2014). Plant extract was prepared by mixing 1 g of oil/leaves in 5 mL distilled water which was then shaked on wrist action shaker for 30 min. The supernatant was removed for checking detoxification efficacy. Sample (1 g) with detected AFB_1 was incubated with 500 µL of different plant extracts for 24 h at 37 °C. After incubation, AFB_1 level of positive sample was quantified again using TLC approach.

Statistical analysis. Data obtained after aflatoxin detection was investigated by applying ANOVA and T paired sample test on SPSS (IBM-SPSS-21). Standard deviation and mean was also calculated. T test is used to determine that whether significant difference was present or not between the means of two groups. Analysis of variance was used to compare two groups. If P value is less than 0.05 result is said to be statistically significant.

Results and Discussion

In this study, aflatoxins in the corn grain and corn flour samples were determined. All the collected samples

contained only AFB_1, other typed of aflatoxins AFB_2, AFG_1, or AFG_2 were not detected in any of the collected sample. AFB_1 has a permissible range of 5 µg/Kg and total aflatoxins have a permissible range of 10 µg/Kg. In overall analysis, AFB_1 was detected in 21 (*n*=21) corn samples of total samples (*n*=80). AFB_1 level in positive samples ranged from 1.51 to 38.54 µg/Kg. Amongst positive samples, AFB_1 level in 13 samples (n=13) was above permissible level, while in 8 samples it was within permissible range *i.e.* up to 2.11 µg/Kg. A total of 26% of the 80 maize (*n*=80) samples tested positive for aflatoxin after analysis. It showed that 62% of contaminated samples were found to be above permissible levels, while 38% were found to be within permissible limits (5 µg/Kg). However, aflatoxins were not found in 74% of the samples. AFB_1 was detected in the highest concentration in corn samples from Lahore. Aflatoxin-producing fungus was found in corn samples collected from fields and warehouses due to improper storage conditions.

Figure 1 area-wise analysis was shown individually, 9 samples (*n*=9) from Kasur and 12 samples (*n*=12) from Lahore were contaminated with AFB_1 out of total 40 corn samples (*n*=40) from each area. Among contaminated samples, 8 samples of Lahore (*n*=8) and 5 samples of Kasur (*n*=5) had aflatoxins beyond permissible range (above 5 µg/Kg). Lh3, Lh5, Lh9, Lh13, Lh16, Lh18, Lh19, Lh20, Lh24, Lh32, Lh37 and Lh40 were all found to be positive in Lahore. Figure 2 shows the quantity of AFB_1 in Lahore samples. Lh24, Lh32, Lh37 and Lh40 were within the permitted range among contaminated samples, but Lh3, Lh5, Lh9, Lh13, Lh16, Lh18, Lh19 and Lh20 were above permissible range.

Corn samples collected from Kasur were less infected by aflatoxins as compared to Lahore. Positive samples from Kasur were K4, K6, K14, K15, K19, K10, K27, K31 and K33. Samples of within permissible range were K10, K27, K31 and K33, while K4, K6, K14, K15 and K19 were beyond safe level. Results of Kasur areas graphically represented in Fig. 3. In current study, it was estimated that samples from lahore were more contaminated by AFB_1, while contamination ratio was lower in Kasoor corn samples as compared to Lahore. AFB_1 was found in higher concentrations 38.54 µg/Kg and 38.08 µg/Kg in sample Lh13 and Lh18 respectively.

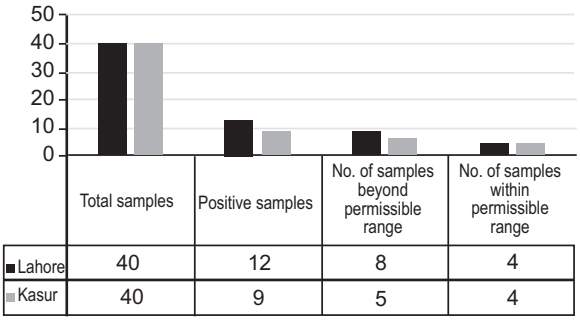

Fig. 1. City wise results of TLC analysis.

Statistical analysis. The average concentration of aflatoxins in Lahore samples was 5.3708 1.75, while the average concentration in Kasur samples was 3.04131.21. As the T test analysis revealed no significant difference between the means of aflatoxin concentration in Lahore and Kasur because the P-value was more than 0.05% (P>0.05%). The P-value in the analysis of variance was 0.95, as it is higher than 0.05 (P>0.05), indicating that the concentration of aflatoxins in both groups was insignificant (Table 1).

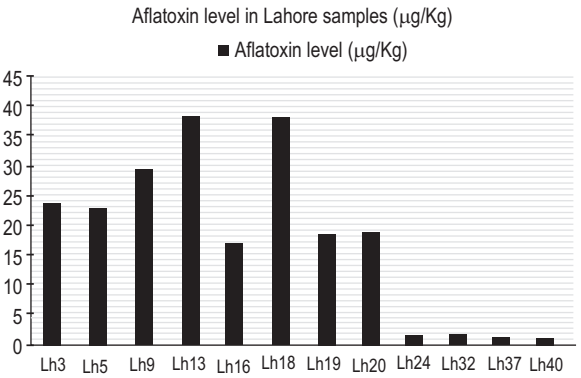

Fig. 2. Aflatoxin concentration in contaminated samples of Lahore.

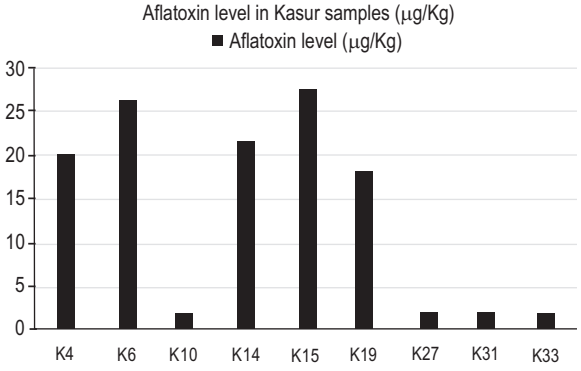

Fig. 3. Aflatoxin concentration in contaminated samples of Kasur.

Table 1. Analysis of variance

Anova					
Aflatoxin concentration in samples collected from Lahore					
	Sum of squares	df	Mean square	F	Sig.
Between groups	435.774	9	48.419	.334	.956
Within groups	4348.610	30	144.954		
Total	**4784.384**	**39**			

Detoxification. Detoxification of AFB_1 was the final step of this study in which AFB_1 was degraded by using different methods *i.e*. physical, chemical and biological.

Detoxification by physical methods. Washing contaminated samples with hot water and simple washing resulted in a 36.6% reduction in AFB_1 compared to washing them with cold water. The highest reduction percentage was shown when Lh13 was subjected to boiling for 10 min at 120 °C. Trend of detoxification by physical methods showed in Fig. 4. It showed boiling was most effective in detoxification as compared to washing.

Detoxification by chemical method. Citric acid, acetic acid, sodium bicarbonate and hydrochloric acid were used for chemical degradation of AFB_1. Lh13 sample with highest contamination was treated with chemical solutions of varying concentration. Citric acid showed remarkable reduction percentage of 52.6% at its 20% concentration. The results showed that 20% citric acid was the most effective in lowering AFB_1, while 5% sodium bicarbonate was the least effective. Figure 5 depicts the overall effectiveness of chemical reagents.

Biological detoxification. Biological degradation or degradation by using natural ways by different plant extracts had showed reduction in AFB_1 level. Plant extracts such as black seed and mustard oil showed maximum reduction in AFB_1 level as compared to ginger and garlic paste. *Brassica juncea* L. oil was most efficient in reducing AFB_1 to 52.1% with decline from 38.08 to 18.22 µg/Kg, while black seed oil removed AFB_1 less than 1%. It showed reduction percentage of 51.5%. Figure 6 showed result of biological degradation of Lh18.

Aflatoxins as name indicates are toxic compounds derived from polyketides produced by certain molds (Hell and Mutegi, 2011). In the present study, 80 samples were gathered from different districts of Lahore. This study was conducted to detect aflatoxin in corn grain and corn flour. The highest level of aflatoxin was found in sample Lh13 with mean value of 38.54 µg/Kg which was much higher than in another study (Majeed *et al*., 2017). In that study different maize varieties in Pakistan were analyzed for aflatoxin detection.

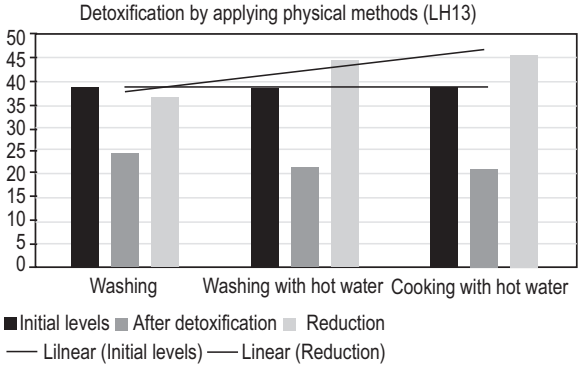

Fig. 4. Detoxification by physical methods.

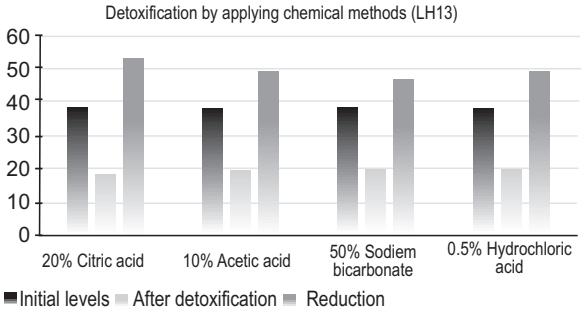

Fig. 5. Detoxification by chemical methods.

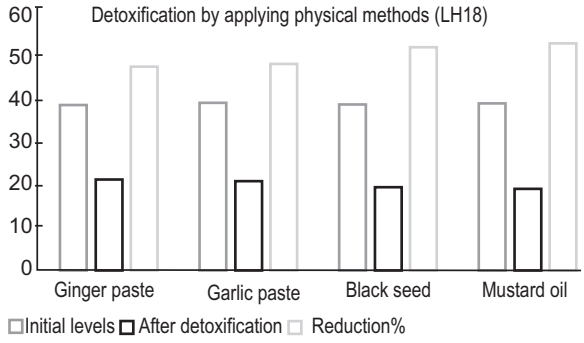

Fig. 6. Detoxification by biological method.

In different ecological zones, a research was conducted on the bio-detoxification of aflatoxins in rice and animal feed. A total of 50 rice samples and 60 feed samples were collected and detoxified using natural substances such as citric acid, sodium bicarbonate, *Allium sativum*, and black seed oil. In the fuming hood, the best conditions for de-toxification were 26 °C for 30 min. *Allium sativum* lowered aflatoxins in corn from 28.5 µg/Kg to 1.42 µg/Kg (a 95% reduction), while black seed oil deactivated aflatoxins from 28.5 µg/Kg to 0 µg/Kg, resulting in a 100% reduction (Nazir *et al*., 2021).

Using a vibrating decontamination apparatus, a study was performed to investigate the effect of UV light on AFB_1 levels in maize and its impact on *A. flavus*. By exposing *A. flavus* to UV rays, it was reduced to 43%, while AFB_1 was reduced to 43%. UV radiation was used on the samples, with doses ranging from 1080 to 8370 mJ/cm (Udovicki *et al.*, 2022). The physical approach for regulating aflatoxins is UV irradiation, whereas the physical agents of control in this investigation were heating and washing. Heating maize at a higher temperature (120 °C for 10 min) resulted in a reduction of 45.7%, whilst washing with hot water resulted in a reduction of 44.3%. When compared to UV irradiation, the percentage reduction achieved higher on heating.

Various investigations have been carried out in order to discover various detoxification methods. In this research, detoxification by various means showed that, while all methods can be used to reduce aflatoxin, detoxification by natural methods is more efficient since it has no negative effects on maize, whereas the use of chemicals can alter the nutritional value of corn. Chemicals used to de-contaminate corn will eventually have negative consequences for animals or people.

The greatest level of aflatoxin was detected in sample Lh13, with a mean value of 38.54 µg/Kg, which was much higher than that found in another study (Majeed *et al*., 2017). In that investigation, aflatoxin levels in various Pakistani maize types were measured. In the desi variety, the greatest mean value of aflatoxin was 14.5 µg/Kg and the results revealed that only the AFB_1 form is the most prominent in all maize types.

Aflatoxins and OTA in corn and rice was identified in a study. Aflatoxin was found in 37 maize samples and 43 corn product samples, with mean values of 5.47 and 7.85 µg/Kg, respectively. A total of 28 maize samples (n=28) were tested for OTA, with a mean value of 5.29 µg/Kg. The detection of OTA and aflatoxins was done using HPLC (Majeed *et al*., 2013). However, only AFB_1 was identified in 21 of the samples (n=21) in my investigation, with levels ranging from 38.54 to 1.51 µg/Kg. None of the aflatoxins other than AFB_1 was detected. The presence of OTA was not observed in this investigation. Instead of HPLC, TLC was utilised in this case. TLC is a faster technique for detecting aflatoxins.

Conclusion

It is concluded that corn samples from different areas of Lahore and Kasur showed variable contamination ratio. The samples of Lahore showed highest aflatoxin incidence as compared to Kasur samples. Corn collected from fields and godowns were more infected by Aspergillus as compared to corn from utility stores. Utility stores have better storage conditions like suitable temperature and controlled humidity. Field samples were more contaminated due to improper handling and storage, varying temperature and high humidity that favoured growth of aflatoxigenic fungus. This study provides recent information regarding aflatoxin contamination of corn in year 2021 in areas of Lahore and Kasur. The research also provides suggestions for the management of food commodities to prevent aflatoxin contamination during harvesting, transport and storage phase. On contamination, different detoxification strategies can be used to degrade affected corn. Among used methods, degradation by biological method is safer as compared to physical and chemical methods.

Conflict of Interest. The authors declare that they have no conflict of interest.

References

Benkerroum, N. 2019. Retrospective and prospective look at aflatoxin research and development from a practical standpoint. *International Journal of Environmental Research and Public Health*, **16:** 3633.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S.D., Haesaert, G., Karlovsky, P., Stroka, J. 2013. Masked mycotoxins: a review. *Molecular Nutrition and Food Research*, **57:** 165-186.

Chavez, R.A., Cheng, X., Stasiewicz, M.J. 2020. A

review of the methodology of analyzing aflatoxin and fumonisin in single corn kernels and the potential impacts of these methods on food security. *Foods*, **9:** 297.

Gacem, M.A., El Hadj-Khelil, A.O. 2016. Toxicology, biosynthesis, bio-control of aflatoxin and new methods of detection. *Asian Pacific Journal of Tropical Biomedicine*, **6:** 808-814.

Heinrich, M., 2003. IARC monographs on the evaluation of carcinogenic risks to humans: some traditional herbal medicines, some mycotoxins, naphthalene and styrene-IARC working group on the evaluation of carcinogenic risks to humans. *Journal of Ethnopharmacology*, **88:** 299-300.

Hell, K., Mutegi, C. 2011. Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, **5:** 459-466.

Holcomb, M., Wilson, D.M., Trucksess, M.W., Thompson Jr., H.C. 1992. Determination of aflatoxins in food products by chromatography. *Journal of Chromatography A*, **624:** 341-352.

Ibrahim, O.O., Menkovska, M. 2018. The nature, sources, detections and regulations of mycotoxins that contaminate foods and feeds causing health hazards for both human and animals. *Journal of Agricultural Chemistry and Environment*, **8:** 33-57.

Jinap, S., De Rijk, T.C., Arzandeh, S., Kleijnen, H.C.H., Zomer, P., Van der Weg, G., Mol, J.G.J. 2012. Aflatoxin determination using in-line immunoaffinity chromatography in foods. *Food Control*, **26:** 42-48.

Khaneghah, A.M., Eş, I., Raeisi, S., Fakhri, Y. 2018. Aflatoxins in cereals: state of the art. *Journal of Food Safety*, **38:** 6.

Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K., Kang, S.G. 2017. Aflatoxins: a global concern for food safety, human health and their management. *Frontiers in Microbiology*, **7:** 2170.

Kumar, V.V. 2018. Aflatoxins: properties, toxicity and detoxification. *Nutrition and Food Science International Journal*, **6:** 555696.

Liu, Y., Wu, F. 2010. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environmental Health Perspectives*, **118:** 818-824.

Mahbobinejhad, Z., Aminian, H., Ebrahimi, L., Vahdati, K. 2019. Reduction of aflatoxin production by exposing *Aspergillus flavus* to CO_2. *Journal of Crop Protection*, **8:** 441-448.

Majeed, S., Iqbal, M., Asi, M.R., Iqbal, S.Z. 2013. Aflatoxins and ochratoxin a contamination in rice, corn and corn products from Punjab, Pakistan. *Journal of Cereal Science*, **58:** 446-450.

Majeed, S., Iqbal, M., Asi, M.R., Iqbal, S.Z., Selamat, J. 2017. Analysis of nutritional traits and aflatoxin contamination in selected maize varieties from Pakistan. *Journal of Food Protection*, **80:** 1993-1998.

Manzoor, M., Farooq, Z., Iqbal, S., Mukhtar, H., Nawaz, M. 2018. Quantification of aflatoxins in maize samples collected from various parts of the Punjab, Pakistan. *JAPS: Journal of Animal and Plant Sciences*, **28:** 6.

Monson, M.S., Coulombe, R.A., Reed, K.M. 2015. Aflatoxicosis: lessons from toxicity and responses to aflatoxin B1 in poultry. *Agriculture*, **5:** 742-777.

Nazir, A., Kalim, I., Imran, M., Bilal, M.A., Zahra, N., Ahmad, A., Iqbal, M., Fazal, U., Ehtisham-ul-Haque, S. 2021. Incidences and bio-detoxification of aflatoxins in rice and cattle feed crops under different agro-ecological zones. *Polish Journal of Environmental Studies*, **30:** 1949-1954.

Nazir, A., Kalim, I., Sajjad, M., Usman, M., Iqbal, M. 2019. Prevalence of aflatoxin contamination in pulses and spices in different regions of Punjab. *Chemistry International*, **5:** 274-280.

Nisa, A., Zahra, N., Hina, S. 2014. Detection of aflatoxins in rice samples. *Bangladesh Journal of Scientific and Industrial Research*, **49:** 189-194.

Pohland, A.E. 1993. Mycotoxins in review. *Food Additives and Contaminants*, **10:** 17-28.

Qureshi, H., Hamid, S.S., Ali, S.S., Anwar, J., Siddiqui, A.A., Khan, N.A. 2015. Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier. *Medical Mycology*, **53:** 409-416.

Rajarajan, P.N., Rajasekaran, K.M., Devi, N.A. 2013. Aflatoxin contamination in agricultural commodities. *Indian Journal of Pharmaceutical and Biological Research*, **1:** 148-151.

Rushing, B.R., Selim, M.I. 2019. Aflatoxin B1: a review on metabolism, toxicity, occurrence in food, occupational exposure and detoxification methods. *Food*

and Chemical Toxicology, **124:** 81-100.

Udovicki, B., Stankovic, S., Tomic, N., Djekic, I., Smigic, N., Trifunovic, B.S., Rajkovic, A. 2022. Evaluation of ultraviolet irradiation effects on *Aspergillus flavus* and aflatoxin B1 in maize and peanut using innovative vibrating decontamination equipment. *Food Control*, **134:** 108691.

Vijayanandraj, S., Brinda, R., Kannan, K., Adhithya, R., Vinothini, S., Senthil, K., Velazhahan, R. 2014. De-toxification of aflatoxin B1 by an aqueous extract from leaves of *Adhatoda vasica* Nees. *Microbiological Research*, **169:** 294-300.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American Journal of Clinical Nutrition*, **80:** 1106-1122.

Zahra, N., Hina, S., Ejaz, N. 2012. Detoxification of aflatoxin B1 in poultry and fish feed by various chemicals. *Pakistan Journal of Scientific and Industrial Research-Biological Sciences*, **55:** 154-158.