Aflatoxins levels in branded and non-branded corn from Lahore, Pakistan

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Abstract

In the present study, presence of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) was checked in branded and non-branded corn samples procured from different areas of Lahore, Pakistan. One hundred and fifty branded and non-branded corn samples were collected in a random manner from 25 different areas (supermarkets, small retail shops, small groceries, and super stores) during May-June 2016 and were analyzed for aflatoxin via thin layer chromatography. The descriptive analysis showed that 64% of the non-branded corn samples were contaminated with three types of aflatoxins *i.e.* AFB_1, AFB_2, and AFG_1, while 23% of the branded corn samples were contaminated with AFB_1 only. In non-branded samples, AFB_1 was detected in the range of 1.25–67.73 µg kg^{-1} followed by AFB_2 (0–8.95 µg kg^{-1}) and AFG_1 (0–16.46 µg kg^{-1}). In branded corn samples, AFB_1 was detected in the range of 1.2-7.07 µg kg^{-1}. Amount of different types of aflatoxins was many times higher as compared to prescribed limit (2 µg kg^{-1}) for the consumption of aflatoxins contaminated food by European Commission. It was concluded that health risks associated with the consumption of such kind of contaminated food may cause carcinogenic diseases, thus, all agricultural samples must be tested for the occurrence of AFs.

Keywords: Aflatoxins, AFB_1, AFB_2, AFG_1, Corn, Thin Layer Chromatography.

Introduction

Aflatoxins (AFs) are naturally occurring and the most toxic class of toxins produced by over 20 species of *Aspergillus* but *Aspergillus flavus, Aspergillus paraciticus* and *Aspergillus nomius* are prominent (Saladino *et al.,* 2016). The acute toxicity and the carcinogenic property of aflatoxins has been recognized for over 40 years (McKean *et al*., 2006). Twenty types of aflatoxins have been identified but only four types of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) are serious concern for public and biologists since last five decades (Juan *et al.,* 2008). These four AFs has been classified on the basis of colors of fluorescence they produce when observed under ultraviolet (UV) light (Dhanasekaran *et al*., 2011). The International Agency for Research on Cancer (IARC) has declared AFB_1, AFB_2, AFG_1 and AFG_2 as carcinogens (IARC, 1993).

AFs enter in human's body and ultimately into the blood by consuming contaminated food or inhaling in the food processing industries having contaminated stored commodities (Yunus *et al.,* 2011). Animals liver like cow, buffalos and goats can metabolize AFB_1 and AFB_2 into less toxic form of AFM_1 and AFM_2 (Nogaim, 2014). However, these toxins cause mortality and reduction in productivity of farm animals. Human liver cannot detoxify AFs, but forms a reactive substance aflatoxin-8, 9-epoxide which binds with DNA to form a DNA adduct. This adduct causes the mutation in p53 gene, a tumor suppressive gene of liver cells ultimately causing cancer (Rawal *et al.,* 2010).

Agriculture crops and stored commodities are susceptible to the infection by *Aspergillus* species and noticeable contamination has been encountered in wheat, rice and corn during harvesting, handling, shipment and storage (Dolezal *et al.,* 2014). Corn, also known as maize (*Zea mays* L.) of family Poaceae is highly nutritious crop. It is cultivated at vast area in India, China, Thailand, and Pakistan. USA is the leader of corn production followed by China and Brazil. In Pakistan, corn production in 2015-16 was 52, 00 thousand metric tons and it contributed 4% to the GDP. The crop is cultivated in all the four provinces of Pakistan in two seasons: a) Kharif maize, planted from Jun–Aug, and b) Rabi maize, planted from Oct-Dec. Punjab and KPK contributes 56% and 39% of total production, respectively (PAR, 2015).

Contrary to cultivation and production, Pakistani farmers use traditional methods of storage which cause spoilage and contamination of corn with AFs. Due to high contamination of AFs in the corn the people in Pakistan are might be at greater risk of cancer. In Pakistan only, it can affect 6 per 100,000 males and 4 per 100,000 females. Most of the patients present in their forties and fifties. It carries

the overall survival rates of 3–5% only (PSSLD, 2013). Due to less number of food testing laboratories and unawareness of public, the amount of AFs in corn is still not detected in Pakistan. The present study was conducted to assess the AF in available branded and non-branded corn samples in the market through thin layer chromatography.

Materials and Methods

Sample collection

Seventy-five samples of each branded and non-branded corn were purchased from supermarkets, small retail shops, small groceries, and specialized suppliers in Lahore, Punjab, Pakistan. All the sample were collected in triplicates and were analyzed under control condition for presence of AFs.

Extraction of aflatoxins

Sample (50 g) was grinded and transferred into 500 mL Erlenmeyer flask. Mixture of 150 mL chloroform and 25 mL distilled water was added into a flask and was subjected to shaking at a frequency of 3 Hz for 30 min with the help of wrist action shaker. After that sample was filtered through Whatman filter paper (No. 42), and filtrate was subjected to evaporation on hot plate at 90ºC for 2 hrs. A thin layer was obtained, which was diluted with 0.5 mL of chloroform. Then 25 µL samples were spotted on thin layer chromatographic plate (TLC). Standard solution of aflatoxins (AFB_1, 2.01 μgL^{-1} AFB_2 0.5 µg L^{-1}, AFG_1 2.01 µg L^{-1} and AFG_2) in 5 µg L^{-1} mL of acetonitrile (CH_3CN) was also spotted on TLC plate for comparison. The plate was kept in TLC tank containing dry ether, and after completion of solvent flow up to the solvent front TLC plates were taken out. The TLC plates was dried and analyzed under Ultra-voile (UV) light.

Confirmation of aflatoxin

The detection of aflatoxins was done by comparing the spots of standard and sample. For positive samples, chromatogram was run in 2nd mobile phase containing chloroform and acetone with ratio of 9:1.

Calculation for amount of aflatoxin

The concentration of aflatoxins in corn samples in µg kg^{-1} was determined by using the given formula:

$$\textbf{Aflatoxins in } \mu\frac{\textbf{g}}{\textbf{kg}} = \frac{\textbf{S x Y x V}}{\textbf{Z x W}}$$

S = Volume of aflatoxins µL in standard solution with intensity equivalent to Z (µL of sample); Y = Concentration of aflatoxins in standard solution µg mL^{-1}; Z = Volume of sample extract in µL required to give the fluorescence intensity compared to Aflatoxin standard S in µL; W = Weight in grams of original sample in final extract.

Statistical analysis

The data collected was subjected to statistical analysis means and standard deviation for better interpretation at 5% probability level by using software Statistix 8.1.

Results and Discussion

Assessment of AFs in branded and non-branded corn samples is summarized in Table 1 and suitability of corn for human consumption is depicted in Fig. 1. It was found that all 75 samples of the non-branded corn were contaminated with AFs, while 64% of the samples were found as unfit with high amounts of AFB_1 (1.25–67.37 µg kg^{-1}). However, AFB_2 (0–8.95 µg kg^{-1}) and AFG_2 (0–16.46 µg kg^{-1}) were detected in 12% and 8% of the non-branded corn samples, respectively. Fewer samples of branded corn (23%) were detected unfit for AFB_1 and its amount was recorded within ranges of 1.2-7.07 µg kg^{-1}. None of the branded corn samples was found to be contaminated with AFB_2 and AFG_2.

About 23% branded and 64% non-branded samples exceeded the permissible limit (2 µg kg^{-1}) fixed for AFs by European legislations (European Commission, 2006) Occurrence of aflatoxins from maize has been reported previously by several workers from different regions (Kos *et al*., 2013; Pleadin *et al*., 2015). Species in the Flavi group are likely to responsible for production of AFs in branded and non-branded corn samples due to prevalence of optimum environmental conditions (25–35 ºC temperature, 0.65 water-activity, and 80% humidity) for their growth and mycotoxin during May to Aug in District Lahore, Pakistan. Besides, traditional techniques like natural drying for storage and mishandling during transportation are being utilized especially in rural areas. During fruit drying process, moisture content decreased and sugar was concentrated resulting in an appropriate growth medium for xero-tolerant molds(Juan *et al*., 2008). Therefore, all non-branded samples were contaminated with AFs. However, branded corn (export quality) is being sold in supermarkets, and branded stores, produced by corn farms, harvested, processed neatly, and stored in good condition. So, fewer amounts of only AFB_1 were detected from these samples as the good storage conditions prohibit the production of AFs in corn (Saleemi *et al*., 2012; Suleiman e*t al*., 2013).

The highest occurrence of AFB_1 might be attributed to contamination of corn samples with AFI producing fungi. *A. flavus* produces B_1 and B_2, and *A. parasiticus* produces B_1, G_1, B_2, and G_2(Hedayati *et al*., 2007). Donner (2009) and Sweany *et al*. (2011) observed *A. flavus* isolates in maize from Kenya and Nigeria, respectively. Horn *et al.* (1995) identified *A. parasiticus* lineages associated with

corn and peanut cultivation in USA, Asia and Africa. Donner (2009) suggested that isolates belongs to *A. parasiticus* together with *A. minisclerotigenes* exhibited tremendous ability to produce aflatoxins in corn. Lai *et al*. (2015) reported 37% strains of *A. flavus* produced AFB_1 and AFB2 among 127 strains.

Conclusions

All corn samples were contaminated with AFs. AFB1 was beyond the permissible limits in 64% of the non-branded and 23% of the branded samples. AFB2 and AFG1 were also detected in non-branded corn samples in 12% and 8% samples, respectively. Serious health risks are associated with the consumption of contaminated corn in Pakistan especially the non-branded corn. The Government of Pakistan must set regulation of testing for every type of corn before circulation into the market.

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Table 1: Occurrence of Aflotoxins in branded and non-branded corn samples.

Types of corn	Types of aflatoxins	No. of Samples	Minimum (µg kg^{-1})	Maximum (µg kg^{-1})	Mean (µg kg^{-1})	Sample (%)	
						Unfit	Fit
Branded	AFB_1	75	1.2	7.07	2.24±0.15	23	77
	AB_2	75	0	0	0±0.00	0	100
	AFG_1	75	0	0	0±0.00	0	100
Non Branded	AFB_1	75	1.25	67.37	11.21±1.75	64	36
	AFB_2	75	0	8.95	0.72±0.24	12	88
	AFG_1	75	0	16.46	1.02±0.42	8	92

The date represents the means ± standard deviation of three replicates. Permissible limits given by (European Commission, 2014) for AFs in corn is < 2.0 µg kg^{-1}.

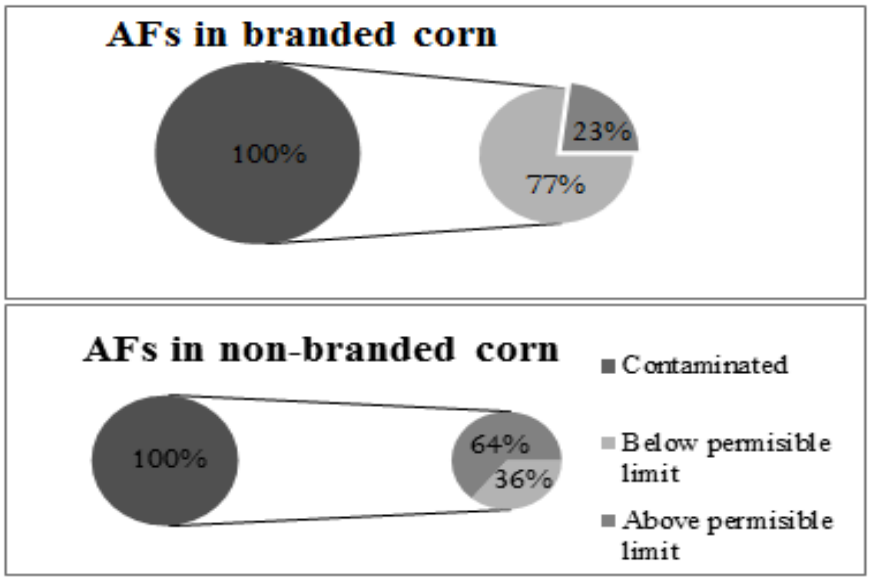

Fig.1: Percentage of fit and unfit corn samples.

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