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OCCURRENCE AND SEASONAL TRENDS OF AFLATOXIN IN RICE, MAIZE AND WHEAT IN BANGLADESH

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ABSTRACT

The study was conducted at the Laboratory of Food Toxicology Research Section, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh. To elucidate the present status of aflatoxin contamination in rice, wheat and maize in Bangladesh, about 180 samples of each commodity were collected throughout the year 2012 in six different times, six Divisions (Dhaka, Rajshahi, Chittagong, Sylhet, Khulna, Barisal) and analyzed by HPLC (High performance liquid chromatography) followed by solid phase extraction for total aflatoxin B_1, B_2, G_1 and G_2. A high incidence, 37 % and a high level of contamination, 280ug/kg was found in maize. Rice and wheat in Bangladesh were not found to have high incidence of aflatoxin. It was also found that in all three commodities' samples collected in July were found to have high moisture content and high incidence rate.

Keywords: Aflatoxin, Occurrence, Contamination, Incidence and Elucidate.

INTRODUCTION

Several dangerous mycotoxins are naturally occurring in foods, feeds, agricultural products and cause health hazards to people and animals. Aflatoxins, the most potent hepatocarcinogen and mutagen among mycotoxins (Hudler, 1998) are produced by many species of *Aspergillus*, a *fungus*, the most notable ones being *Aspergillus flavus* and *Aspergillus parasiticus*. However, *A. flavus* has been described as a new species, *A. nominus* (Kurtzman *et al.,* 1987). At least 14 different types of aflatoxins are produced in nature. The main aflatoxins are B_1, B_2, G_1 and G_2. The European Union set action levels for food grains and feed stuffs (commission regulation 466/2001). Chronic, subclinical exposure does not lead to symptoms as dramatic as acute aflatoxicosis. Children are particularly affected by aflatoxin exposure, which leads to stunted growth and delayed development (Abbas, 2005). Chronic exposure also leads to a high risk of developing liver cancer (Aguilar *et al.,* 1993). The expression of aflatoxin-related diseases is influenced by factors such as species, age, nutrition, sex, and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver (Machida and Gomi, 2010). Low levels of aflatoxin exposure require continuous consumption for several weeks to months to develop signs of liver dysfunction to appear (Bingham *et al.,* 2003). Aflatoxins have been isolated from all major cereal crops. The staple commodities regularly contaminated with aflatoxins include maize, rice, wheat, and a variety of spices intended for human or animal consumption (Rambo *et al.,* 1975; Stoloff, 1976; Qutel *et al.,* 1983; Pozzi *et al.,* 1995; Ewaduh, 1992; Adebajo *et al.,* 1994; Duttor and Westlene., 1985). When processed, aflatoxins get into the general food chain where they have been found in both pet and human foods, as well as in feed stocks for agricultural animals. International sources of commercial peanut butter, cooking oils (i.e. olive oil, etc) and cosmetics have been identified to be contaminated with aflatoxin. In many of these contaminated food products, the aflatoxin exceeded FDA or other regulatory agency, safe limits (McDaniel, 2012; Leong *et al.,* 2012; Mahoney *et al.,*2010; Li *et al.,* 2009). When aflatoxin B_1 is ingested some transformations occur and secondary new aflatoxin M_1 and M_2 having same acute toxicity as B_1 is produced which are generally found in cow's milk (Coker, 1979). Occupational exposures to aflatoxins in agricultural workers, people working in oil mills, and granaries have been reported (Sorenson *et al.,* 1984). After large experimentation on many animal species like rats, rainbow trout's, aflatoxin especially aflatoxin B_1 is confirmed as a potential carcinogen (IARC, 1993). Metabolism plays a major role in deciding the degree of toxicity (Eaton *et al.,* 1994). Species susceptibility to aflatoxin mainly depends on its liver detoxification systems, genetic makeup, age and other nutritional factors (Ramdell *et al.,* 1990. A study in West Africa showed a

significant correlation among the aflatoxin exposure and stunted growth in children who are exposed to aflatoxin right for neonatal stages (Gong *et al.,* 2002). Apart from that due to the capacity of aflatoxins to cross the placental barrier, can cause genetic defects at foetal stages itself (Maxwell *et al.,* 1998). In Bangladesh, rice, maize and wheat are reported to be aflatoxin contaminated but the present status is not known (Mustafa *et al.*2000; Huq *et al.,* 1999; Dawlatana *et al.,* 2002). The objective of this study was to elucidate the present status and seasonal trends of aflatoxin contamination in rice, wheat and maize in Bangladesh.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Food Toxicology Research Section, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh.

Sampling: Rice, maize and wheat were collected and analyzed to carry out this work. Collection site of six divisions namely Dhaka, Rajshahi, Chittagong, Sylhet, Khulna and Barisal of Bangladesh, numbers and weights of samples taken in six different times of the year 2012 are outlined in table 1. A total of 180 samples of each commodity were analyzed for total Aflatoxin B_1, B_2, G_1 and G_2.

Table 1. Samples of rice, maize and wheat were collected from six divisions Dhaka, Rajshahi, Chittagong, Sylhet, Khulna and Barisal.

Sample collection area	January (No. and weight)	March (No. and weigh)	May (No. and weight)	July (No. and weight)	September (No. and weight)	November (No. and weight)	Total (No. of samples
CSDs (Central storage depots) / Mill sample	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	60
Markets	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	60
Farmer's stores	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	10x 5 kg	60

For bag storages (like Central Storage Depots, CSDs) each 5kg sample was composed of fifty 100 grams increments. For markets ten stalls were randomly selected and 500 grams were purchased to make one composite sample of 5kg. From randomly selected ten villagers one composite 5 kg sample was purchased from village open market which was brought by the farmer to sell as a representative sample of their store. The 5kg was sub-divided in to 1kg sub-sample in a rotary Cascade sample divider (Pascall Engineering Co. Ltd., England) and powdered in a sub-sampling mill by Simplabelt Variable speed drive (Simplatroll Ltd., Bedford) to collect 200 gram representative sample.

Apparatus: Blender (Waring commercial, England), Mechanical shaker - flatbed, Denley Ins., SPE vacuum manifold, (Supelco Visiprep 5-7030). SPE reservoirs -70 ml and 30 mL, (Varian), SPE adaptors (Varian), SPE Cartridge-3 mL, 500 mg PH packing, Bond-Elut (Varian), Sample concentrator, Techne, DB-3, Syringe filter cartridge -13 mm diameter x 0.45 micron, Disposable Syringes -3 mL, Volumetric flask - 250 mL and 10 mL. Vortex mixer, Remi Equip., CM101, Micro syringe capable of injection volumes up to 50 microliters and HPLC system (Agilent).

Reagents: Acetone, Methanol and Acetonitrile (HPLC grade), sodium sulfate anhydrous (heated for at least 2 hour at 550°C), Lead acetate, chloroform. All the solvents used for the analysis was purchased from Merck, Germany. Aflatoxins standards were obtained from Sigma Chemicals, USA.

Sample preparation

Extraction: Extraction was carried out from 200 gram sub-sample by making slurry with water at 1:2 ratio of sample: water. An appropriate volume of acetone was added to 100 gram of slurry to produce acetone to water ratio 1:4 and shaked in a mechanical shaker for 30 minutes and then collected the filtrate through a Whatman no. 1 filter paper in a conical flask. 10 ml methanol and 1 ml lead acetate added to 10 ml of the filtrate in a 250 ml measuring cylinder and made up to 150 ml with distilled water (Huq *et al.,*1999).

Clean up: Clean up was done using SPE Cartridge 3 mL, 500 mg PH packing was attached to 75 ml reservoir and a vacuum manifold. The cartridge was conditioned by passing of 15 mL methanol followed by 15 ml water under vacuum after adding 1 gram methanol washed celite. Then 150 ml prepared sample solution was passed through cartridge under vacuum at the rate of 10 ml/ min. The cartridge was then washed with 10 ml of water. Any remaining water from the cartridge was removed by the passage of air for about 5 minutes. The 75 ml reservoir was replaced with a 25 ml reservoir and successively with another reservoir (4 ml) containing anhydrous sodium sulphate (500 mg) and inserted between the cartridge and vacuum manifold. The aflatoxins were eluted using 4 ml of chloroform at the rate of 0.5 ml/min in a 7 ml vial (Huq *et al.,* 1999). The vial was dried under the stream of nitrogen at 45°C in a sample concentrator and reconstituted with 1 ml methanol and water (50:50) for HPLC analysis.

Sample analyses

Samples were analyzed using HPLC system-Agilent: Liquid chromatography consist of Agilent : Solvent delivery system (pumps) Series 1100, Agilent series 1100 Column oven Agilent 1200 series Flourosence detector, Manual injector and Cobra cell for post column derivatization. Software: Agilent ChemStation. HPLC Column was C18, 250mm (L) X 4.6mm (ID) 10µ (Grace). Mobile phase was 630 ml water, 220 ml methanol, 150 ml acetonitrile, 120 uL of concentrated Nitric acid and 100 mg potassium bromide in isocratic mode with 1 ml flow rate. Total run time was 15 minute and Injection volume was 20 uL. Column oven temperature was 30° C and excitation wavelength 365 nm, emission wavelength 464 nm. Recovery was calculated of aflatoxins (B_1, B_2, G_1 and G_2) fortified at 2 ug/kg, 10 ug/kg, 20 ug/kg, 100 ug/kg and 200 ug/kg levels using peak area of chromatograms at concentrations ratio 5:1 (B_1, G_1: B_2, G_2) of standards and was found 87-92 %. Suitable seven point calibration curve was done, preferably on matrix at 0.5, 2, 10, 25, 50, 100 and 250 ng/ml (ug/kg) level. Linear regression was 0.99. The calibration batch was prepared from the mixed working standard of 1000 ug/kg in methanol and water (1:1) which was prepared from stock standard of 20 ppm in acetonitrile. A control spiked samples of 2 ug/kg, 50 ug/kg and 200 ug/kg was run after every 10 samples followed by a solvent as blank. The method was validated as per European commission decision (Commission decision, 2002). The detection limit was 0.5 ug/kg, Decision Limit (CCα) was 4.34 ug/kg and Detection Capability (CCβ) was 4.64 ug/kg.

RESULTS AND DISCUSSION

Results

A total of about 180 samples of each commodity of rice, maize and wheat were collected and all were analyzed for total aflatoxin (B_1, B_2, G_1 and G_2). All the results were shown in the table 2a, 2b, 3a, 3b, 4a and 4b. From the table 2a and 2b, it was found that rice samples collected from CSDs, markets and farmer's store were contaminated with a maximum of 90ug/kg, 124ug/kg and 241ug/kg respectively moisture content were a maximum of 17%, 19% and 20% respectively. The incidence rates were 15%, 23% and 20% respectively.

Table 2a. Moisture content and level of total aflatoxin contamination in rice collected in six different times of 2012.

Time of sample collection	CSDs samples		Market sample		Farmer's stored sample	
	Moisture content (%)	Level of total aflatoxin (ug/kg)	Moisture content (%)	Level of total aflatoxin (ug/kg)	Moisture content (%)	Level of total aflatoxin (ug/kg)
January	9 - 11	-	10- 12	12	9 - 12	6
March	9 - 13	8	10 - 14	108	10 - 13	124
May	10 -13	93	11 - 14	2 - 120	10 - 14	109
July	10 - 17	2-90	12 - 19	5 - 124	11 - 20	2 - 241
September	10- 14	1 - 65	10 - 14	6 - 87	10 - 14	2 - 131
November	10 -14	108	10 - 13	1 - 94	10 - 14	17

Table 2b. Incidence rate of aflatoxin contamination in rice.

Time of sample collection	CSDs samples			Market sample			Farmer's stored sample		
	Number of sample	Samples contaminated	Incidence rate (%)	Number of sample	Samples contaminated	Incidence rate (%)	Number of sample	Samples contaminated	Incidence rate (%)
January	10	-	-	10	1	10	10	1	10
March	10	1	10	10	1	10	10	1	10
May	10	1	10	10	3	30	10	1	10
July	10	3	30	10	5	50	10	6	60
September	10	3	30	10	2	20	10	2	20
November	10	1	10	10	2	20	10	1	10
Total	60	9	15	60	14	23.3	60	12	20

Table 3a. Moisture content and level of total aflatoxin contamination in maize collected in six different occasions of 2012.

Time of sample collection	Mill samples		Market sample		Farmer's stored sample	
	Moisture content (%)	Level of total aflatoxin (ug/kg)	Moisture content (%)	Level of total aflatoxin (ug/kg)	Moisture content (%)	Level of total aflatoxin (ug/kg)
January	10 -15	8 - 163	10- 15	4 - 142	11 - 14	2 - 94
March	11- 15	13 - 93	10 - 15	12 - 85	11 - 16	17 - 184
May	10 - 16	4 - 154	11 - 15	3 - 237	10 - 16	10 - 165
July	10 -20	27 - 169	11 - 21	40 -255	12 - 23	6 - 280
September	11 -15	7- 28	11 - 15	9 - 177	10 -18	10 - 196
November	10 - 14	204	10 - 15	5 - 144	10 - 16	18 - 138

Table 3b. Incidence rate of aflatoxin contamination in maize.

Time of sample collection	Mill sample			Market sample			Farmer's stored sample		
	Number of sample	Samples contaminated	Incidence rate (%)	Number of sample	Samples contaminated	Incidence rate (%)	Number of sample	Samples contaminated	Incidence rate (%)
January	10	2	20	10	3	30	10	3	30
March	10	2	20	10	2	20	10	3	30
May	10	4	40	10	3	30	10	4	40
July	10	6	60	10	5	50	10	7	70
September	10	2	20	10	3	30	10	2	20
November	10	1	10	10	2	20	10	3	30
Total	60	17	28.3	60	18	30	60	22	36.6

In the table 3a and table 3b, it was found that maize samples collected from Mills of North Bengal, markets and farmer's store were contaminated with a maximum of 204 ug/kg, 255 ug/kg and 280 ug/kg respectively, moisture contents were at a maximum of 20%, 21 % and 23 % respectively. The incidence rates were 28 %, 30 % and 36 % respectively. More than 14 % moisture content also was observed in the maximum maize sample. It was found from table 4a and 4b that wheat samples collected from CSDs, markets and farmer's store were contaminated with a maximum of 166 ug/kg, 250 ug/kg and 198 ug/kg respectively, moisture contents were at a maximum of 17%, 20% and 23 % respectively. The incidence rates were 17%, 23% and 22% respectively.

Table 4a. Moisture content and level of total aflatoxin contamination in wheat collected in six different times of 2012.

Times of sample collection	CSDs samples		Market sample		Farmer's stored sample	
	Moisture content (%)	Level of total aflatoxin (ug/kg)	Moisture content (%)	Level of total aflatoxin (ug/kg)	Moisture content (%)	Level of total aflatoxin (ug/kg)
January	10 -11	107	10- 13	3 -109	10 - 13	3- 82
March	11 -13	3 -166	10 - 15	81	10 -14	12 - 192
May	11 -13	9 -128	10 -14	13 -100	10 -15	106
July	10 -17	5 -157	11 -20	1 -250	11 -23	4 -198
September	10 -14	122	10 - 14	2 -175	10-15	5 - 85
November	11 - 3	47	10 -14	26 -85	11 -13	2 - 108

Table 4b. Incidence rate of aflatoxin contamination in wheat.

Times of sample collection	CSDs samples			Market sample			Farmer's stored sample		
	Number of sample	Samples contaminated	Incidence rate (%)	Number of sample	Samples contaminated	Incidence rate (%)	Number of sample	Samples contaminated	Incidence rate (%)
January	10	1	10	10	2	20	10	2	10
March	10	2	20	10	1	10	10	2	10
May	10	2	20	10	3	30	10	1	10
July	10	3	30	10	4	40	10	4	60
September	10	1	10	10	2	20	10	2	20
November	10	1	10	10	2	20	10	2	10
Total	60	10	16.6	60	14	23.3	60	13	21.6

Discussion

In this study, it has been found that in all three commodities samples collected in July were found to have high moisture content and high incidence rate. This may be due to (June-August) wet season of Bangladesh which is favorable for fungal growth. Relatively higher temperature with high moisture content is conducive to aflatoxin contamination. Moreover, Bangladesh is a tropical country with hot and humid climate which makes a suitable environment for the growth of moulds. Studies were conducted to assess the fungal flora associated with different food grains. With paddy varieties (Mia *et al.,* 1986) reported to have 19 fungi belonging to 17 genera which included moulds as well as seed-borne pathogens. It was found that maize was highly contaminated and may be transferred to cattle and poultry feed as it is a main source of feed. Among the three major foods, the staple food rice and wheat in Bangladesh was not to have found high incidence of aflatoxin. Though huge amount of wheat is imported by this country every year; no data were available about imported and home-grown wheat. It was also found that market samples were highly contaminated than the farmer's stored samples. It happened due to lack of proper storage condition. Bangladesh government has no data on aflatoxin contamination in the food of its people. Proper knowledge regarding aflatoxin, its bad effect on our health and economy and also good preservation technique of food and food grains should be disseminated among the mass people, stockholders and farmers. Scientific seminar and workshop can also be arranged. The country should establish quality control limits for certain commodities intended for export or import because diet is the major way through which humen as well as animals are exposed to aflatoxins.

CONCLUSION

This study was carried out under the research and development programme of Bangladesh Council of Scientific and Industrial Research (BCSIR) to find out the present status and seasonal trends of aflatoxin in rice, maize and wheat. It is good that no high incidence of aflatoxin contamination was found in rice and wheat but maize was the commodity most susceptible to aflatoxin contamination. There is a chance of aflatoxin to get in to the human food chain through poultry feed. So, an awareness programme should be taken in Bangladesh.

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