MYCOFLORA AND MYCOTOXIN DETECTION IN ROASTED PEANUT KERNELS OF BANGLADESH

M. M. Khandaker*, T. Rahman[1], M. M. Rahim[1], M. T. Hassan[1], M. Begum[1] and M. A. B. Bhuiyan[2]

**Department of Botany, Jagannath University, Dhaka, Bangladesh.*
[1]IFST, BCSIR, Dhaka, Bangladesh,
[2]BSMRAU, Gazipur, Bangladesh
***Corresponding author:** maniruzzamanbot@gmail.com

ABSTRACT

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Ten samples of roasted peanut kernels were collected from different areas of Bangladesh for determination of mycoflora and mycotoxin association. Peanut kernels were cultured on PDA and blotter. Fungi associated with the tested samples throughout the investigation were *Aspergillus flavus, Aspergillus niger, Aspergillus* sp. (1), *Aspergillus* sp. (2), *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp. and *Curvularia* sp. A total of 3630 fungal colonies were formed on both the media. Among these fungi the highest percentage of colonies (39.12%) were formed by *Aspergillus flavus* followed by *A. niger* (23.75%) and the lowest percentage of colonies (1.68%) were formed by *Curvularia* sp. The maximum number of fungal colonies (566) was found in sample 1 and the minimum number of colonies (148) was found in sample 5. *A. flavus*, *A. niger* and *Penicillum* sp. were commonly present in all the ten samples of peanut kernels. The highest 73.91% of the kernels of sample 1 were found to be infected while in sample 5 it was 25.17%. Fungal colonies grown on PDA were higher in number than blotter in four samples, and colonies formed on blotter were higher in number in six samples. Out of ten roasted peanut samples four were detected with various amounts of aflatoxins (B1, B2, G1, G2). The highest 38.94 ppb of total aflatoxins were detected in sample 02 and the lowest 5.67 ppb of total aflatoxins were found in sample 01. No aflatoxin was detected from six samples.

Key words: Roasted peanut, mycoflora, *Aspergillus flavus* and aflatoxin.

INTRODUCTION

The peanut (*Arachis hypogaea* L.) also known as the groundnut, is a species in legume crop, mainly grow for its edible seeds. Peanut seed kernels are used to make processed food such as tasty dry roasted peanut, peanut butter, oil etc. It is one of the major components of products being manufactured by the food processing industries. Roasted peanut kernels were found to be rich in antioxidants as that of blackberries and strawberries (Talcott *et al*. 2005, Win *et al.* 2011). Roasted peanuts contain 21.51 g of carbohydrates per 100 g. The major carbohydrate present in peanuts is starch. When starch undergoes enzymatic degradation in the body by the action of amylase, it is initially broken down to maltose and isomaltose (Zeeman *et al.* 2010). These two disaccharides, when subjected to respective digestive enzymes, are converted to glucose and release energy. Peanuts contain all the essential amino acids necessary for normal body growth and metabolism. Peanut contains at least 13 different types of vitamins and also rich in 26 essential minerals. Peanuts are a good source of zinc. Zinc is an essential mineral for normal growth and development during pregnancy, childhood, and adolescence. One hundred grams of roasted peanuts provide 3.31 mg of zinc (Settaluri *et al.* 2012). Per 100gm peanuts contain 2.26 mg of iron. It is involved in oxygen transport and helps regulate cell growth and differentiation. It is known that the deficiency of iron leads to anemia which is prevalent in many parts of the world (Miret *et al*. 2003). The fungi characteristic of a region or special environment is called mycoflora. Peanut seeds are good substrate for growth and subsequent aflatoxin production by aflatoxigenic fungi (Xue *et al*. 2003). A major challenge in peanut production is fungal and aflatoxin contamination.

Mycotoxins are known to be hazardous to the health of humans, in some cases directly causing illness and even death. About 10 compounds present a known toxicological impact on human and animal health (Wu *et al*. 2011). Mycotoxins are mainly produced by fungal species belonging to the genera *Aspergillus, Penicillium* and *Fusarium* which are ubiquitous in the environment. Aflatoxins are first isolated and characterized from *Aspergillus flavus*, this mould is a common contaminant of poorly stored

grains (Klich 2007). Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. Aflatoxins have received most attention due to their significance in agricultural production loss, threats to human health and potential threats to food safety. There are roughly 20 known aflatoxins but only four of these (aflatoxins B1, B2, G1 and G2) are studied because of their toxic effects. The International Agency for Research on Cancer (IARC) has reported that aflatoxins, especially aflatoxin B1 are the most potent natural carcinogenic substances and are being linked to severe illnesses and also increase the risk of liver cancer in humans (Anon. 1993). Roasted peanuts are being used as food directly and/or in various forms and are very popular to all sects of people of Bangladesh. But mycoflora and mycotoxin in roasted peanuts are not well documented in Bangladesh. So, the present investigation was aimed to determine the mycoflora and mycotoxin in roasted peanut from different areas of Bangladesh.

MATERIALS AND METHODS

A total of ten roasted peanut samples were collected from different areas of Bangladesh for determination of mycoflora and mycotoxin association (Table 1).

Table 1. List of roasted peanut samples collected from different areas of Bangladesh

Sample Number	Collection Area	Total No. of samples	Collection Date
01	Nawabpur	1	22.01.2016
02	Bhoirob	1	25.04.2016
03	Munshiganj	1	10.05.2016
04	Tangail	1	27.05.2016
05	Gazipur	1	28.05.2016
06	Narayangonj	1	28.05.2016
07	Shahbagh	1	29.05.2016
08	Keranigonj	1	10.06.2016
09	Joypurhat	1	25.06.2016
10	Dinajpur	1	14.07.2016

Each of the ten collected samples was thoroughly mixed and divided into two units and each unit contained 200 g of kernels, where one unit is tested for mycoflora identification and another was tested for mycotoxin detection. Half of the unit for mycoflora detection was grown on blotter and half on PDA. In blotter method peanut kernels of each sample were selected randomly. Peanut kernels were surface sterilized with 10% sodium-hypo-chloride (NaOCl) for one minutes and rinsed thrice in sterile distilled water. After surface sterilization the peanut kernels were air dried and were put in three layered sterilized water soaked blotting paper in Petri-dishes (90mm dia) at the rate of 6 seeds per plate. The plates were kept at room temperature (22-25 C) for four days. Similarly, another set of peanut kernels of each sample were placed in PDA plates as it was mentioned for blotter plates. The colonies developed on the culture media were counted. For single colony, the isolation was done by transferring a bit of mycelial tip or a single spore into the medium containing sterile PDA for further study. The morphology of fungal colonies developed on culture medium was studied and identified by observing characteristics of colony, spore, conidiophore, mycelia etc. (Ellis 1971, Barnett and Hunter 1972, Ellis 1985).

Peanut kernels were analyzed by HPLC (High Performance Liquid Chromatography, Model No. Agilent: 1100 series) by the method of Food Toxicology Research Section of Institute of Food Science Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka which is validated according to the EC decision 2002/657/EC.

HPLC condition:

a. Mobile phase =Acetonitrile: Methanol: Water (Fig. 3. A).
   = 22.5: 22.5: 55
b. Column: C-18,25cm × 4.6 mm (10µm packing).
c. Flow rate: 1.5 ml/min.
d. Column temperature: 30°C.
e. Injection volume: 20 µl.
f. Detector: Fluorescence Detector (Agilent, G 1321A).
g. Excitation Wavelength: 365 nm.
h. Emission Wavelength: 418 nm.
i. Software: Agilent Chem Station for 3D systems. Rev. A. 02.

RESULTS AND DISCUSSION

A total of 3630 colonies were formed in both PDA and blotter media by the eight fungi of five genera namely *Aspergillus flavus*, *A. niger*, *Aspergillus* sp 1, *Aspergillus* sp 2, *Curvularia* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. (Fig. 3 and 4). The highest number of colonies (39.12%) was formed by *A. flavus* followed by *A. niger* (23.75%) and the lowest 1.68% colonies were formed by *Curvularia* sp.

The maximum number of fungal colonies occurred in sample 1 (566) followed by sample 9 (497) and the minimum number of colonies occurred in sample 5 (148) showing their corresponding occurrence of 15.60%, 13.70% and 4.11%, respectively. Among the fungal genera *A. flavus*, *A. niger* and *Penicillum* sp. were commonly found in all the ten samples of groundnut (Table 2).

Table 2. Fungal colonies formed on PDA and blotter by ten roasted peanut kernels and their percent occurrence

Sample	Number of fungal colony								Total	% Occurrence
	A.F	A.N	A1	A2	Pen	Fu	Rz	Cur		
01	200	96	178	-	35	45	03	09	566	15.60
02	80	68	-	-	57	-	28	23	256	7.10
03	71	284	-	-	26	36	-	05	422	11.63
04	201	54	105	-	19	-	-	05	384	10.58
05	60	38	-	-	15	26	09	-	148	4.11
06	193	88	-	-	43	23	09	-	356	9.81
07	85	116	-	73	16	08	-	03	301	8.30
08	145	35	-	31	40	35	13	07	306	8.42
09	188	29	-	-	204	45	22	09	497	13.70
10	197	54	106	-	36	-	01	-	394	10.85
Total	1420	862	389	104	491	218	85	61	3630	
%	39.12	23.75	10.72	2.87	13.52	6.00	2.34	1.68		100

A.F= *Aspergillus flavus,* A.N= *Aspergillus niger*, A1= *Aspergillus* sp. (1), A2= *Aspergillus* sp. (2), Cur= *Curvularia* sp. Fu= *Fusarium* sp. Pen = *Penicillium* sp. Rz= *Rhizopus* sp.

The number of fungal colonies varied among various samples as well as the culture media. Number of fungal colonies formed on PDA were higher in four samples (Samples 4, 8, 9 and 10) whereas number of colonies formed in blotter were higher number in the samples 1, 2, 3, 5, 6 and 7 (Fig. 1).

Percentage of fungal infection in peanut samples varied sharply among the locations. Kernels of sample 01 were mostly infected with fungal flora and colonies were developed from 73.91% kernels, while 26.09% kernels were uninfected i.e. no colony was formed from them. Similarly, 60.19% kernels were infected in sample 02 and 39.81% kernels were uninfected. Kernels of sample 5 were mostly (74.83%) uninfected (Fig. 2).

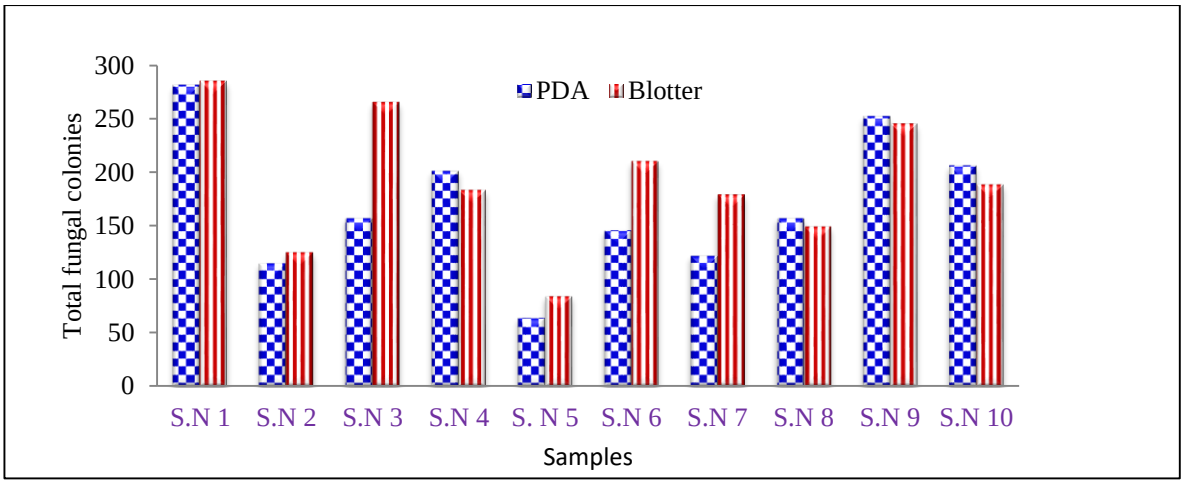


Fig. 1. Total fungal colonies of ten roasted peanut samples formed in two specific (PDA and blotter) media

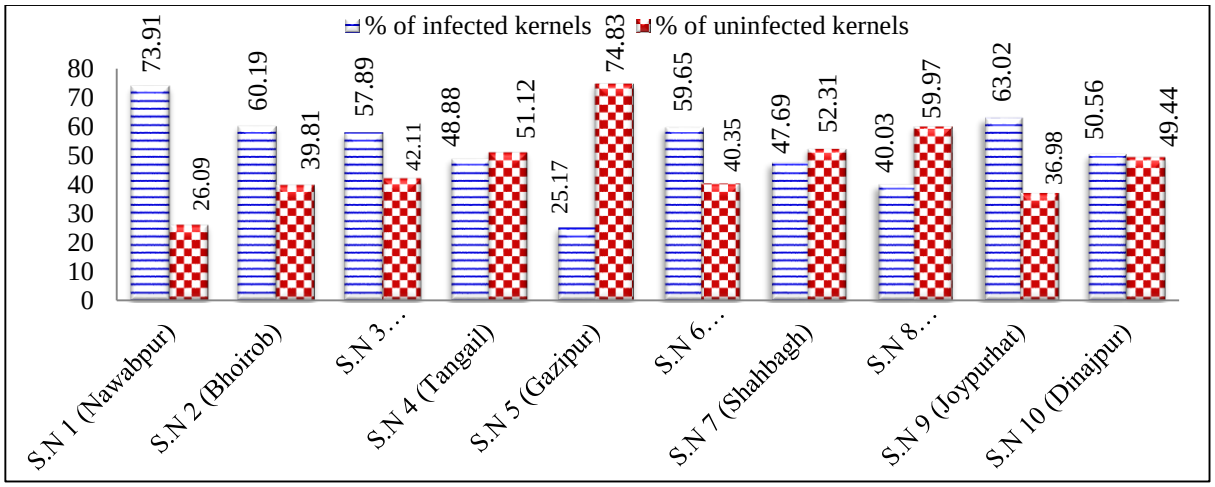


Fig. 2. Percentage of infected and uninfected kernels of ten roasted peanut samples

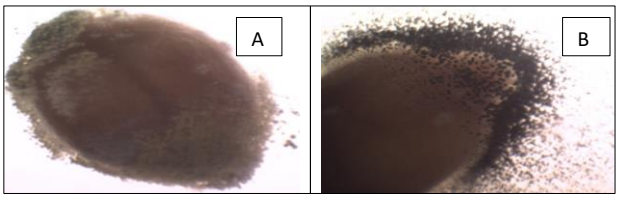


Fig. 3. Stereomicroscopic photograph of fungal genera in roasted peanut kernels. A) *Aspergillus flavus.* B) *Aspergillus niger*

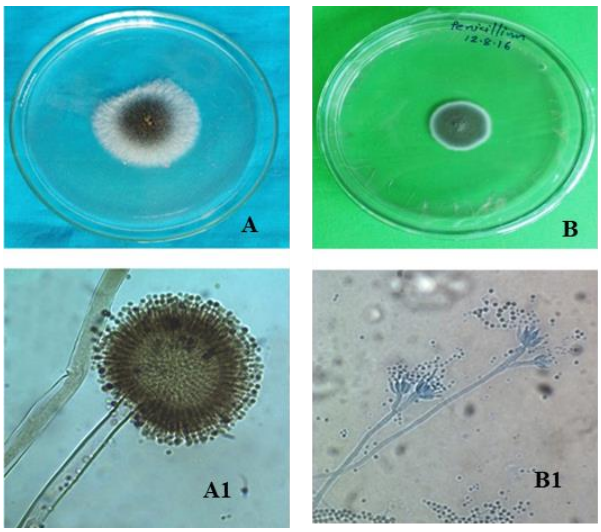


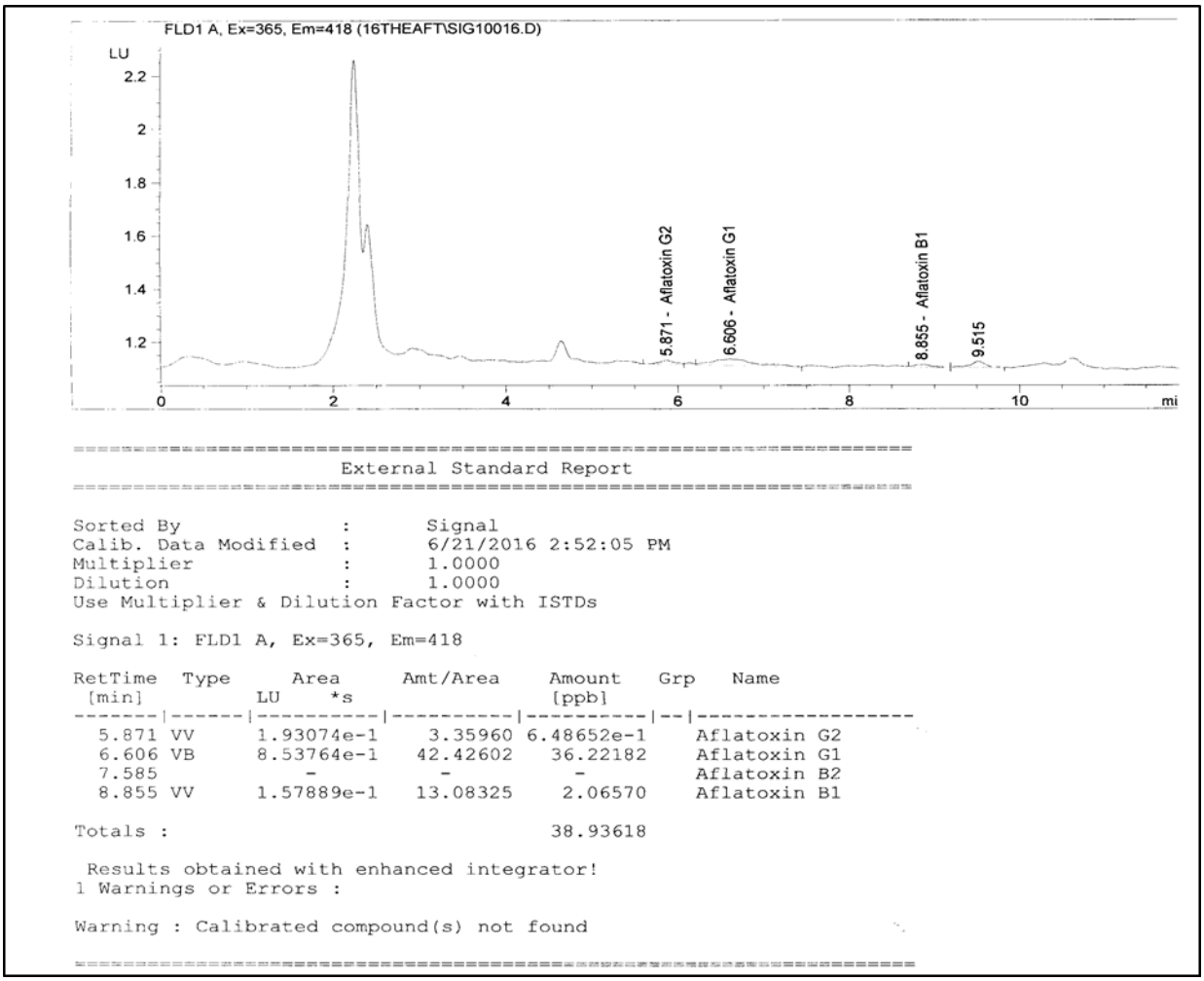
Fig. 4. Pure culture (A. *Aspergillus niger*, B. *Penicillium* sp. and microscopic view (A1. *Aspergillus niger*, B1 *Penicillium* sp.) of two fungi associated with peanut kernel.

Table 3. Aflatoxin analysis result of roasted peanut samples

Sample No.	Aflatoxins (ppb)				Total amount of aflatoxins (ppb)
	B1	B2	G1	G2	
01	5.67	-	-	-	5.67
02	2.07	-	36.22	0.65	38.94
03	5.64	0.23	-	-	5.87
08	5.27	-	-	1.22	6.49

Maximum Residue Limit (MRL): a) Aflatoxin B1: 2 ppb, b) Aflatoxin B1, B2, G1, G2: 4ppb. As per Commission Regulation (EC) no. 165/2010

The highest 38.94 ppb of total aflatoxins were detected in sample 02, which including aflatoxin B1= 2.07 ppb, G1= 36.22 ppb, G2= 0.65 ppb and aflatoxin B2 was not detected. From sample 08 total aflatoxins 6.49 ppb were detected which including aflatoxin B1= 5.37ppb, and G2= 1.22 ppb. In this sample aflatoxin B2 and aflatoxin G2 were not detected. From sample 03 total aflatoxins 5.87 ppb were detected, which were included aflatoxin B1= 5.64 ppb, and B2= 0.23 ppb. Aflatoxin G1 and aflatoxin G2 were absent in this sample. The lowest 5.67 ppb of total aflatoxins were detected from sample 01, which including only aflatoxin B1= 5.67 ppb and other type of aflatoxins were absent. Aflatoxin B1 was present in all of the four aflatoxin detected samples. No aflatoxins was detected from the samples 04, 05, 06, 07, 09 and 10 (Table 3, Fig. 5).



External Standard Report

Sorted By : Signal
Calib. Data Modified : 6/21/2016 2:52:05 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime [min]	Type	Area LU *s	Amt/Area	Amount [ppb]	Grp	Name
5.871	VV	1.93074e-1	3.35960	6.48652e-1		Aflatoxin G2
6.606	VB	8.53764e-1	42.42602	36.22182		Aflatoxin G1
7.585		-	-	-		Aflatoxin B2
8.855	VV	1.57889e-1	13.08325	2.06570		Aflatoxin B1

Totals : 38.93618

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Figure 5. Detection of aflatoxin (G1, G2, B1 and B2) in peanut kernel samples

From the investigation of roasted peanut samples, a large number of fungal colonies were formed in blotter technique than the PDA media. Nearly similar results were found by Nagpurne and Patwary (2014). They worked on the seed mycoflora of different varieties of groundnut seed screened by ISTA. In a previous study, it was shown that peanut roasting and de-coating processes reduce the fungal population in kernels and risk of aflatoxin production (Galvez *et al.* 2003). Khandaker *et al.* (2018) observed total aflatoxin with the range of 11.91 to 182.6ppb in raw peanut kernels of Bangladesh. Results of this study supported the earlier findings. The present results of four detected roasted samples indicated that amount of aflatoxin B1 and amount of total aflatoxins (B1, B2, G1, G2) exceeded the European Union permissible limits of 2 ppb and 4 ppb, respectively (Commission Regulation No. 165/2010). It is very essential to regulate peanut aflatoxin and also create awareness among mass people specially producers and traders.

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