RESEARCH ARTICLE

Determination of Mycoflora and Mycotoxins in Raw and Roasted Peanuts in Bangladesh

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Abstract

Mycoflora and mycotoxin contamination are major challenges in peanut (*Arachis hypogaea* L.) production, trading and health concern to humans and animals. A total of 24 peanut samples out of which 12 were raw and 12 were roasted; were collected from different places in Bangladesh. These samples were examined for detection of mycoflora and mycotoxin. The mycoflora associated with the peanuts belonged to 11 fungal genera such as *Aspergillus, Alternaria*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Mucor*, *Penicillium*, *Pestalotia*, *Rhizopus* and *Trichoderma* and frequencies of occurrence ranged from 0.33% to 71.95%. *Aspergillus* (71.95%) was the most dominant while the least was *Trichoderma* (0.33%). The fungal genus *Aspergillus* was found to be the most occurred (5501 colonies) and *Pestalotia* was the least occurred (25 colonies) pathogen in all samples. The highest number of fungal colonies (393) were formed in raw peanut collected from Sadarpur, Faridpur, however, the lowest number of fungal colonies (232) were formed in raw peanut collected from Potuakhali. *Aspergillus* was available in all the 24 samples. *Penicllium* was present in 12 raw peanut samples; on the other hand, *Rhizopus* was present in 12 samples of roasted peanuts. Aflatoxins were detected from 5 raw samples and 3 roasted samples and detection level of total aflatoxin ranged from 1.72 to 8.52 µg kg^{-1}. The highest level of aflatoxin (8.52 µg kg^{-1}) was detected in raw peanuts from Sibchor, Madaripur. Maximum amount of total aflatoxin among the roasted peanut was 6.71 µg kg^{-1} collected from Chorvodrasan, Faridpur district of Bangladesh. Finding of this study would help us in planning strategies for awareness and management of aflatoxin.

Key words: Aflatoxin, *Aspergillus,* Mycoflora, Peanut.

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Introduction

Peanut (*Arachis hypogaea* L.) also known as groundnut is one of the species in the family Leguminaceae. It is widely grown at tropical and sub-tropical regions. It contains up to 50% oil, 28% protein, and is a rich source of dietary fibre, minerals and vitamins (Nigam *et al*., 2004). It also contains 6% nitrogen, 1% phosphorus, 1% potash, sodium and magnesium. With the increasing cost of animal protein, peanuts have become an important source of protein in the world. In Bangladesh, the peanut production is 33000 MT/year as of 2008 (FAO, 2008). As human food, peanut is used in the form of raw, boiled or roasted nuts, as edible oil and protein by being pounded and used as a vegetable oil for cooking, or made into paste and eaten with sweet potatoes, cassava, banana among others dishes. For cash, peanut is sold in the local market as boiled unshelled and shelled roasted nuts while some are sold in the confectionery trade. Different types of fungi were found to be associated with peanut shells and kernels. Fungal species commonly isolated from the peanut sample included *Aspergillus flavus* L. strain, *A. flavus* S strain, *A. parasiticus, A. tamarii, A. caelatus, A. alliaceous* and *A. niger.* Fungi isolated in low frequency included *Fusarium* spp., *Penicillium* spp., *Mucor* spp., *Rhizopus* spp. (Nyirahakizimana *et al*., 2013). Seven fungi such as *Aspergillus flavus, A. parasiticus, Fusarium moniliforme, F. solani, Macrophomina phaseolina, Rhizoctina solani* and *Sclerotium rolfsi* have been frequently isolated with different frequencies from either pod shells or seeds of peanut (Mahmud, 2004).

In a study, Passone *et al.* (2009) observed that *A. flavus* was the most frequently isolated species from peanut. This species produces mycotoxins. Mycotoxins are substances which produced mostly as secondary metabolites by filamentous fungi that grow on seeds, grains, and feed in the field, or in storage. Maize and peanuts are the main sources of human exposure to aflatoxin because they are highly consumed worldwide. These crops are the most susceptible to aflatoxin contamination (Wu and Khlangwiset, 2010).

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In Bangladesh aflatoxins were detected in eight peanut samples out of 13 samples at the range of 11.91 to 182.61 ppb (Khandaker *et al*., 2018). Factors responsible for the high incidence of aflatoxin contamination of peanuts include poor agricultural practices during planting, harvesting, drying, transportation and storage of the product which helps fungal growth (Coulombe, 1991). Several types of aflatoxin exist, but the four main types are aflatoxin B1, B2, G1 and G2, with aflatoxin B1 being the most toxic (Olaru *et al*., 2008). While both *A. flavus* and *A. parasiticus* can produce the B toxins, *A. parasiticus* which is more prevalent in peanuts than in other crops, also produces the G toxins (Diener *et al*., 1987). Aflatoxin contamination escalates when peanuts are stored in a facility where there is a poor air circulation in the immediate environment (Mutegi *et al*., 2013).

Pascale and Visconti (2008) have summarized the various methods available for mycotoxin analysis including Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography/Mass spectrometry (LC/MC), Enzyme-Linked Imunosorbent Assay (ELISA), and rapid tests. Though peanuts and

its products are popular to the people of Bangladesh, but studies on peanut mycoflora and mycotoxin are lacking. So, this investigation was carried out to detect the peanut mycoflora association and mycotoxin level in peanut collected from different regions of Bangladesh.

Materials and Methods

Raw and roasted peanut samples were collected from farmers, local and retail sources of different places in Bangladesh (Table 1). Each of the collected samples was divided into two parts and each part containing 250 g of peanuts; one part was used for mycoflora determination and other part was kept for mycotoxin detection. International Seed Testing Association (ISTA, 1966) standard was maintained. Each sample containing 250 g of peanuts were surface sterilized by sodium hypo-chloride and then rinsed with distilled water. Then the peanuts were placed on the sterile blotting paper to soak extra water and samples were air dried. Then 15 ml of sterilized liquid Potato Dextrose Agar (PDA) was poured on sterilized plate. The plates were kept for few minutes for solidification of the medium. In the mean time, from the air dried samples shells and kernels were separated. Six shells and kernels were placed into the plate on PDA separately. The plates were kept at 20-22C temperature for few days. Then the samples were examined under a stereomicroscopic (SnEu 2112657) and/or microscope for the determination of mycoflora and their frequencies. Fungi which were grown on the plates were identified with the help of standard references (Barnett and Hunter, 1972; Alexopoulos *et al*., 2014). For a single colony, the isolation was done by transferring the fungal hyphal tip or a single spore into the medium containing sterile PDA.

Table 1: Places, nature and collection date of peanut samples from different parts of Bangladesh.

Sample no.	*Nature*	*Collection area*	*Collection date*
1	Raw	Bhanga, Faridpur	10/07/16
2	Raw	Sadarpur, Faridpur	10/07/16
3	Raw	Chorvodrasan, Faridpur	10/07/16
4	Raw	Shibchor, Madaripur	15/07/16
5	Raw	Fotulla, Narayongonj	25/06/16
6	Raw	Barura, Comilla	01/06/16
7	Raw	Kishorgong	05/06/16
8	Raw	Potuakhali	11/06/16
9	Raw	Shantipur, Barishal	17/07/16
10	Raw	Mirpur, Dhaka	29/11/16
11	Raw	Savar, Dhaka	03/12/16
12	Raw	Uttara, Dhaka	29/12/16
13	Roasted	Bhanga, Faridpur	10/07/16
14	Roasted	Sadarpur, Faridpur	10/07/16
15	Roasted	Chorvodrasan, Faridpur	10/07/16
16	Roasted	Shibchor, Madaripur	15/07/16
17	Roasted	Fotulla, Narayongonj	25/06/16
18	Roasted	Barura, Comilla	01/06/16
19	Roasted	Kishorgonj	05/06/16
20	Roasted	Potuakhali	11/06/16
21	Roasted	Shantipur, Barishal	17/07/16
22	Roasted	Mirpur, Dhaka	29/11/16
23	Roasted	Savar, Dhaka	03/12/16
24	Roasted	Uttara, Dhaka	29/12/16

Peanuts were analyzed by HPLC (High Performance Liquid Chromatography, Model No. Agilent: 1100 Series) following the method of Association of Official Analytical Chemists (AOAC, 2007), for detection of aflatoxin. All of the 24 samples were included for mycotoxin analysis.

Results and Discussion

The occurrence of seed borne fungi associated with peanut varied in number among the 24 samples collected from different areas of Bangladesh. In this study, the results indicated that the frequencies of fungal colonies were ranged from 0.33 % to 71.95%. A total of 11 fungal genera were observed with following frequencies viz., *Aspergillus* (71.95%), *Alternaria* (0.94%), *Chaetomium* (3.61%), *Cladosporium* (2.54%), *Curvularia* (1.02%), *Fusarium* (0.46%), *Mucor* (3.44%), *Penicillium* (5.85%), *Pestalotia* (0.37%), *Rhizopus* (9.51%) and *Trichoderma* (0.33%).

The fungal genus *Aspergillus* was found to be associated with all the collected samples at maximum occurrence of 5501 fungal colonies. Occurrence of other fungal colonies in genera was: *Rhizopus* (727), *Penicillium* (447), *Chaetomium* (276), *Mucor* (263), *Cladosporium* (194), *Curvularia* (78), *Alternaria* (72), *Fusarium* (35), *Pestalotia* (28) and *Trichoderma* (25). The highest 393 colonies was formed in sample 2 followed by 385 colonies in sample 4, and the lowest 232 colonies was formed in sample 8. *Aspergillus* colonies were formed in all the raw and roasted peanut samples (samples 1-24). *Penicillium* was present in all raw peanut samples (samples 1-12). *Alternaria* was present only in sample 2 and *Curvularia* in sample 5 of raw peanuts. *Aspergillus* and *Rhizopus* were present in all the 12 samples of roasted peanuts (samples 13-24). *Penicillium* was grown in 10 roasted samples. No colony was formed by *Pestalotia* and *Trichoderma* in any of the roasted samples whereas *Fusarium* was present in one sample (sample 24) of roasted peanut (Table 2).

Total number colonies formed in raw peanuts were 3864 and in roasted peanuts 3782. Infection frequencies by raw and roasted peanut samples were almost same and frequencies were 50.54% and 49.46%, respectively. *Aspergillus*, *Chaetomium*, *Mucor* and *Penicillium* were higher in number in raw peanut samples, on the other hand *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium* and *Rhizopus* were higher in roasted peanuts (Table 3). Likewise, Rashed *et al*. (2004) isolated 14 genera and 28 species of fungi; among these *Aspergillus flavus*, *A. niger, Penicillium* sp., and *Fusarium* sp. were found predominant. Fungi *A. flavus, A. parasiticus, F. moniliforme, F. solani* have been frequently isolated with different frequencies from either pod shells or seeds of peanut (Mahmud, 2004). Vinay and Patwari (2014) also found 7 genera and 12 species of fungi; among these fungi *Aspergillus flavus*, *Aspergillus niger, Fusarium* sp. and *Alternaria* sp. were predominant and shows higher percentage of seed mycoflora.

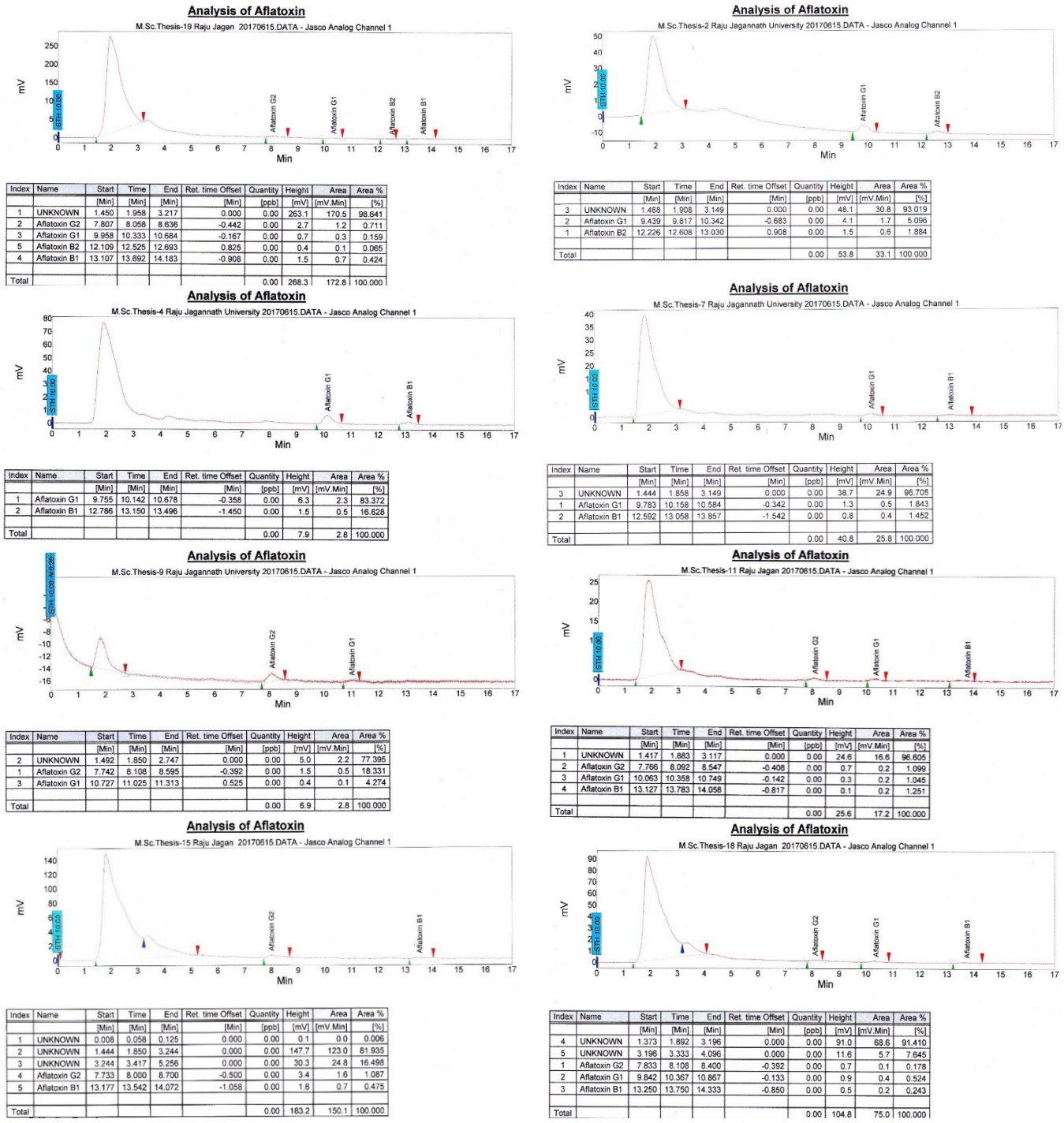
In this study all the samples were analyzed for aflatoxins by High Performance Liquid Chromatography (HPLC). Results showed that a total of eight samples (33.33%) were positive for total aflatoxin at levels of 1.72 to 8.52 µg kg^{-1}. Out of 12 raw peanuts samples aflatoxin was detected from 5 samples (sample 2, 4, 7, 9 and 11) and out of 12 roasted peanut samples three (sample 15, 18 and 19) were infected by aflatoxins. The highest (8.52 µg kg^{-1}) total aflatoxin was detected from sample 4 of raw peanuts followed by 6.75 µg kg^{-1} from sample 2 of raw peanuts. The lowest 1.72 µg kg^{-1} total aflatoxin was detected from sample 11 of raw peanuts. Maximum amount of total aflatoxin among the roasted peanut samples was

Table 2: Mycoflora colonies associated with the raw (samples 1-12) and roasted (samples 13-24) in peanut samples.

	Total number of colonies in peanut samples																								
Fungal genera	*S-1*	*S-2*	*S-3*	*S-4*	*S-5*	*S-6*	*S-7*	*S-8*	*S-9*	*S-10*	*S-11*	*S-12*	*S-13*	*S-14*	*S-15*	*S-16*	*S-17*	*S-18*	*S-19*	*S-20*	*S-21*	*S-22*	*S-23*	*S-24*	*Total*	*Frequency (%)*
Aspergillus	298	236	274	221	258	150	209	180	242	271	240	258	201	217	206	235	208	278	298	182	260	219	157	203	5501	71.95
Alternaria	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	9	-	26	19	-	72	0.94
Chaetomium	-	-	12	25	8	107	-	11	-	32	8	-	-	-	-	-	-	-	-	-	32	17	-	24	276	3.61
Cladosporium	-	-	-	-	-	-	-	-	-	20	56	-	-	-	-	-	-	-	-	65	14	17	22	-	194	2.54
Curvularia	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	-	18	-	-	17	22	78	1.02
Fusarium	-	7	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18	35	0.46
Mucor	-	38	-	33	17	-	18	15	20	-	-	16	52	10	-	-	19	-	25	-	-	-	-	-	263	3.44
Penicillium	23	28	43	44	15	22	3	26	13	9	17	23	40	14	8	20	11	13	-	-	19	12	14	30	447	5.85
Pestalotia	10	-	-	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	0.37
Rhizopus	15	69	-	41	-	-	86	-	-	-	23	-	30	33	33	55	67	56	29	44	22	32	27	65	727	9.51
Trichoderma	-	15	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	0.33
Total	346	393	339	385	308	279	316	232	293	332	344	297	323	274	247	310	312	358	352	318	347	323	256	362	7646	100

Table 3: Associated mycoflora colonies with raw (samples 1-12) and roasted (samples 13-24) peanut samples.

			Frequency (%) of Raw and Roasted samples	
Genera of peanut mycoflora	*Colonies in Raw (samples 1-12)*	*Colonies in Roasted (samples 13-24)*	*Raw*	*Roasted*
Aspergillus	2837	2664	50.54	49.46
Alternaria	11	61		
Chaetomium	203	73		
Cladosporium	76	118		
Curvularia	10	68		
Fusarium	17	18		
Mucor	157	106		
Penicillium	266	181		
Pestalotia	28	0		
Rhizopus	234	493		
Trichoderma	25	0		
Total	3864	3782		



Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
1	UNKNOWN	1.450	1.958	3.217	0.000	0.00	263.1	170.5	98.641
2	Aflatoxin G2	7.807	8.058	8.636	-0.442	0.00	2.7	1.2	0.711
3	Aflatoxin G1	9.958	10.333	10.684	-0.167	0.00	0.7	0.3	0.159
5	Aflatoxin B2	12.109	12.525	12.693	0.825	0.00	0.4	0.1	0.065
4	Aflatoxin B1	13.107	13.692	14.183	-0.908	0.00	1.5	0.7	0.424
Total						0.00	268.3	172.8	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
3	UNKNOWN	1.468	1.908	3.149	0.000	0.00	48.1	30.8	93.019
2	Aflatoxin G1	9.439	9.817	10.342	-0.683	0.00	4.1	1.7	5.096
1	Aflatoxin B2	12.226	12.608	13.030	0.908	0.00	1.5	0.6	1.884
Total						0.00	53.8	33.1	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
1	Aflatoxin G1	9.755	10.142	10.678	-0.358	0.00	6.3	2.3	83.372
2	Aflatoxin B1	12.786	13.150	13.496	-1.450	0.00	1.5	0.5	16.628
Total						0.00	7.9	2.8	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
3	UNKNOWN	1.444	1.856	3.149	0.000	0.00	38.7	24.9	96.705
1	Aflatoxin G1	9.783	10.158	10.584	-0.342	0.00	1.3	0.5	1.843
2	Aflatoxin B1	12.592	13.058	13.857	-1.542	0.00	0.8	0.4	1.452
Total						0.00	40.8	25.8	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
2	UNKNOWN	1.492	1.850	2.747	0.000	0.00	5.0	2.2	77.395
1	Aflatoxin G2	7.742	8.108	8.595	-0.392	0.00	1.5	0.5	18.331
3	Aflatoxin G1	10.727	11.025	11.313	0.525	0.00	0.4	0.1	4.274
Total						0.00	6.9	2.8	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
1	UNKNOWN	1.417	1.883	3.117	0.000	0.00	24.6	16.6	96.605
2	Aflatoxin G2	7.766	8.092	8.547	-0.408	0.00	0.7	0.2	1.099
3	Aflatoxin G1	10.063	10.358	10.749	-0.142	0.00	0.3	0.2	1.045
4	Aflatoxin B1	13.127	13.783	14.058	-0.817	0.00	0.1	0.2	1.251
Total						0.00	25.6	17.2	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
1	UNKNOWN	0.008	0.058	0.125	0.000	0.00	0.1	0.0	0.006
2	UNKNOWN	1.444	1.850	3.244	0.000	0.00	147.7	123.0	81.935
3	UNKNOWN	3.244	3.417	5.256	0.000	0.00	30.3	24.8	16.498
4	Aflatoxin G2	7.733	8.000	8.700	-0.500	0.00	3.4	1.6	1.087
5	Aflatoxin B1	13.177	13.542	14.072	-1.058	0.00	1.6	0.7	0.475
Total						0.00	183.2	150.1	100.000

Index	Name	Start [Min]	Time [Min]	End [Min]	Ret. time Offset [Min]	Quantity [ppb]	Height [mV]	Area [mV.Min]	Area % [%]
4	UNKNOWN	1.373	1.892	3.196	0.000	0.00	91.0	68.6	91.410
5	UNKNOWN	3.196	3.333	4.096	0.000	0.00	11.6	5.7	7.645
1	Aflatoxin G2	7.833	8.108	8.400	-0.392	0.00	0.7	0.1	0.178
2	Aflatoxin G1	9.842	10.367	10.867	-0.133	0.00	0.9	0.4	0.524
3	Aflatoxin B1	13.250	13.750	14.333	-0.850	0.00	0.5	0.2	0.243
Total						0.00	104.8	75.0	100.000

Fig. 1: Aflatoxins analysis results of eight detected samples (Samples 2, 4, 7, 9, 11, 15, 18 and 19) by HPLC method.

6.71 µg kg^{-1} recorded in sample 15. The rest 16 samples of which 7 raw and 9 roasted samples were free from aflatoxin (Table 4, Fig. 1).

The mycotoxins produced by *Aspergillus* spp. is the greatest significance in peanut (*Arachis hypogaea*) and peanut products include aflatoxins and ochratoxins (Pittet and Pittet, 1998). Aflatoxin B1, B2, G1 and G2 are produced by some strains of *Aspergillus flavus*, *A. parasiticus*, *A. nomius* (Cotty and Bhatnagar, 1994). *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Alternaria* sp. species that often contaminate foodstuffs and feed-stuffs and produce most of the mycotoxins that threaten humans and animals. Pascale and Visconti (2008) have summarized High Performance Liquid Chromatography as very important method for mycotoxin analysis. The fungi *Aspergillus* spp. association with seed is a likely to be a threat in storage as it rises its infection under improper storage conditions. Hence, there is a need for reducing the mold growth and mycotoxin production by improving the cultural, harvesting, storage and processing conditions.

Table 4: Mycotoxin (Aflatoxin) analysis results on raw and roasted peanut samples from different places in Bangladesh by HPLC method.

Sample no.	*Nature of samples*	*Aflatoxins (µg kg^{-1})*				*Totalaflatoxins (µg kg^{-1})*
		B1	*B2*	*G1*	*G2*	
1	Raw	-	-	-	-	ND
2		-	1.27	5.48	-	6.75
3		-	-	-	-	ND
4		1.11	-	7.41	-	8.52
5		-	-	-	-	ND
6		-	-	-	-	ND
7		0.89	-	1.61	-	2.50
8		-	-	-	-	ND
9		-	-	0.32	1.61	1.93
10		-	-	-	-	ND
11		0.44	-	0.64	0.64	1.72
12		-	-	-	-	ND
13	Roasted	-	-	-	-	ND
14		-	-	-	-	ND
15		1.55	-	-	5.16	6.71
16		-	-	-	-	ND
17		-	-	-	-	ND
18		0.44	-	1.29	0.32	2.05
19		1.55	-	0.97	3.87	6.60
20		-	-	-	-	ND
21		-	-	-	-	ND
22		-	-	-	-	ND
23		-	-	-	-	ND
24		-	-	-	-	ND

Conclusion

Mycoflora associated with 24 collected raw and roasted peanut samples from Bangladesh were belonging to 11 genera, viz. *Aspergillus, Alternaria*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Mucor*, *Penicillium*, *Pestalotia*, *Rhizopus* and *Trichoderma*. Among these *Aspergillus* was the most predominant with 71.95% occurrence. *Aspergillus* was observed in all 24 peanut samples and number of colonies formed was 5501. *Penicllium* was present in 12 raw peanut samples and *Rhizopus* was present in 12 roasted peanuts samples. Aflatoxins were detected from five raw samples and three roasted samples and detection level of total aflatoxin raged from 1.72 to 8.52 µg kg^{-1}. The highest (8.52 µg kg^{-1}) total aflatoxin was detected from sample 4 of raw peanuts.

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