Chapter

Aflatoxins and Fumonisins Contamination of Maize in Bangladesh: An Emerging Threat for Safe Food and Food Security

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Abstract

Maize (Bhutta) is one of the important growing cereal crops in Bangladesh. Toxigenic fungi such as *Aspergillus* and *Fusarium* infect stored maize grains. Enzyme-linked immusorbent assay (ELISA) was used to determine total aflatoxins and fumonisins contamination in stored maize grains collected from 15 Bangladeshi maize-producing areas. The highest total concentration of aflatoxins (103.07 µg/kg) and fumonisin (9.18 mg/kg) was found in Chuadanga and Gaibandha, whereas the lowest was detected for aflatoxins (1.07 µg/kg) and (0.11 mg/kg) in Dinajpur and Cumilla, respectively. The findings clearly demonstrated that aflatoxin concentrations in samples from six regions and fumonisin concentrations in samples from 10 regions were beyond the regulatory limit of aflatoxin (10 ppb) and fumonisin (1 ppm), respectively, as set by European Union (EU). However, a positive correlation between aflatoxins with toxigenic *A. flavus*, and fumonisins with toxigenic *Fusarium* spp. was observed. The fungi associated with maize grains were identified by sequencing of ITS regions. Moreover, toxigenic *A. flavus* was confirmed using primers specific to *nor*, *apa2*, *omtA* and primer FUM1 for *F. proliferatum* and *F. oxy sporum*. Since the Bangladesh Food Safety Authority has not authorized any precise regulation limits for maize mycotoxin contamination, these results will serve as a benchmark for monitoring mycotoxin contamination in maize and also to develop globally practiced biocontrol approach for producing safe food and feed.

Keywords: mycotoxins, maize, threat, food, security

1. Introduction

Maize (Bhutta) or *Zea mays* L. (corn) is one of the supreme vital cereals in the globe which belongs to Poaceae family and it has been ranked as a third position in the last few decades after wheat and rice [1]. A fair number of food and industrial commodities such as maize flour, animal feed, cooking ingredient, corn syrup, grain alcohol and whiskey are processed from maize [2]. Maize has been known as a significant emerging crop in Bangladesh as well as maize production is familiarized
day by day due to its diverse use for feed, food, fish meal and edible oil processing [3]. Bangladesh has achieved 11th position when it comes to average yield which was 8 tons per ha in the year of 2019–2020 [4] and maize production were 40 lakh ton [5]. Anyway, maize plant is quite vulnerable for various fungi as they get favorable environment to infect via fluctuation of humidity and temperature conditions in both of storage and growing phase [6]. In harvesting period less care in drying and storage processing leads to a surge in infection and production of toxin [7]. Dominant pathogens such as Aspergillus spp. and Fusarium spp. in maize have the capability to destroy seeds, germination procedure in seeds as well as generating vital mycotoxins [8]. Mycotoxins are light molecular weight developed from saprophytic fungi, most significantly Aspergillus, Fusarium and Penicillium as secondary metabolites [9]. Mycotoxins were detected as one of the deadly toxins after the outbreak of ruinous ‘Turkey X’ in 1960s at England which leads to the death of Turkey poults (100,000) [10]. Mycotoxin contamination can develop in any stage of food chain especially in the field, during transportation, processing, harvesting and storage [11].

Aflatoxins are mainly hepatocarcinogenic toxins comprising of major three metabolites named Aflatoxin G, M and B under derivative compounds named difurocoumarin [12–14]. The paramount aflatoxin producing fungi globally is A. flavus divided into two distinct morphotypes named L and S [15], among them S morphotype was potentially ruinous as it was capable of producing gigantic level of toxins [16, 17]. A significant research has been made by toxigenic communities that innumerable lineages of fungi are belong to S morphotype among them a few were able to engender enormous concentration of both B and G aflatoxins [18]. Several Aspergillus spp. is accounted for several toxins such as aflatoxin B is mainly produced from A. flavus, A. parasiticus whereas aflatoxins G is developed from A. nomius. Moreover, G and B are highly produced inspices, fruits, corn, nuts, peanuts and copra [19, 20]. A. flavus is ubiquitous and mostly detected in corn producing toxins, while in peanut A. parasiticus is the main culprit of developing toxins [21]. The toxicity level of aflatoxins of different types chronologically are B1 > G1 > B2 > G2 [22]. Basically, aflatoxins levels were found ascendency in the food markets of Bangladesh [23]. Temperature, pH, relative humidity, and the presence of other fungi are predominant factor for developing aflatoxins and substrates [24]. Aflatoxins level surges due to drought, insect damage, and heat during fungal growth [25]. The AflR gene regulates the activation of other structural genes including omt-A, ver-1, and nor-1, which are involved in the aflatoxin biosynthesis process [26]. In hot and humid settings, aflatoxins contamination are also thrived [27]. Seasonal variation has been observed in Bangladesh including high humidity, high temperature and seasonal variation in rainfall (http://en.wikipedia.org/wiki/Geography of Bangladesh). Extreme humid conditions significantly triggered the growth of aflatoxins [28], as a result, it is obvious that aflatoxins was reported in maize, cereals and groundnuts and other feed in Bangladesh and exceeding European Union (EU) permissible limit for aflatoxins [29].

Fusarium spp. are among the utmost crucial fungal pathogens of maize, where they cause severe abatement of yield and accumulation of a vast range of harmful mycotoxins in the grain [30]. Fusarium spp. also have the ability to infect crucial crops such as potato, wheat, barley, asparagus, mango, oats, rice and other feed and food crops [31]. High moisture conditions triggered the production of Fusarium toxins near or at harvesting stage in cereals [32, 33]. Fumonisins toxins can be developed from a numerous species such as F. moniliforme, F. verticillioides, F. nygamai, F. proliferatum [34] as well as A. niger [35]. Fumonisins comprise of four types of toxins which are A, B, C, and P, among them fumonisin B1 is the most exploited and ruinous one [34]. FB1, FB2, and FB3 were designated as utmost
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destructive and highly abundant fumonisin toxins where FB1 is the most ruinous due to its availability of high concentration on host ranging from 70 % to 80 % of all fumonisins [36–38]. Several biotic (temperature, water stress) and abiotic (osmotic stress, pH, and fungicides) factors are responsible for Fusarium growth and Fumonisin production [39, 40]. At maturity stage damage occurs by insects, during flowering wet warm weather, rain before harvest, humidity, and media composition for both the Fusarium spp., all the activities are related to fumonisins production [41, 42]. FUM1 gene can also expressed by ecological conditions reported by [43, 44]. As Fusarium is widespread and ubiquitous in all cereal growing regions of the globe and corresponding mycotoxins are produced which has been influenced by storage methods and crop production [45]. In the midst of milling, storage, processing, cooking of food and feed, Fusarium are highly stable due to its structure and humans and animals are exhibited to them to a certain degree [46–48]. In Bangladesh, animal feed samples were detected and found fumonisin contamination mainly maize based feed contamination [49].

An investigation came out that in South Asia has been ranking as the utmost prevalent continent in case of exposing aflatoxins contamination (82 %) in the globe as well as 41 % maize samples were detected higher amount of aflatoxins contamination than the permissible limit of lenient EU criteria [49]. The very first outbreak of mycotoxin (Sterigmatocystin) was found in Bangladesh in rice straw [50], later in maize and poultry birds [51]. Liver cancer and hepatitis B infection promotes carcinogenic potency in specific individuals by aflatoxins [52, 53]. In Japan, in the year of 1991–2009, violation cases were detected exceeded 1500 in foods which were imported at a level of 10–4918 mg/kg [54]. 62 % children with the age of 3 are at a complete risk of infecting with aflatoxins as aflatoxins biomarkers was detected in plasma of their blood [55]. According to WFP (World Food Program), permissible limit of aflatoxins is 10 ppb (10 μg/kg) and for fumonisins it is 1 ppm (1 mg/kg) [56]. Fumonisins toxin may causes esophageal carcinoma in humans [57], as well as contaminated with folate uptake in cellular level [58] and surging the intensity of neural tube defect [59]. 52 % positive rate of fumonisins was found with an overall level of 936 mg/kg in Asia [60]. Fusarium mycotoxin can cause leukoencephalomalacia, porcine pulmonary edema and rat hepatocarcinoma in human and livestock as well [55, 61, 62] detected that in Dhaka, Bangladesh 62 % of 3 year old children had aflatoxin biomarkers in their blood plasma revealing chronic aflatoxin exposure as reported earlier that significant amount aflatoxins were found from corn selling in the Bangladeshi market. Probably 1311 cases of liver cancer was detected every year in Bangladesh [63]. In can be deduced from abovementioned fact that determining aflatoxins and fumonisins and all other mycotoxins in food and feed are the prime need for the country like Bangladesh as these mycotoxin substantially subverts our plants yield concurrently human and animal lives as well. Thus, more research needs to be conducted to elicit the specific mycotoxin hampering specific food, feed and plants, besides to find out the plausible management for controlling these mycotoxins. This study highly exhibited the aflatoxins and fumonisins toxin level in Bangladesh from maize samples of different regions as it has been concerned as one of the burning issues for ensuring safety food.

2. Materials and methods

2.1 Sample collection

Composite stored maize grain samples were collected from 15 maize growing areas of Bangladesh such as Bogura, Kushtia, Meherpur, Chuadanga, Kishoreganj, Manikganj, Cumilla, Rajshahi, Dinajpur, Rangpur, Natore, Thakurgaon,
Panchagarh, Nilphamary and Jashore. Maize samples were collected from stores of traders in local markets of different districts. Ten markets were sampled in each district having at least five traders in each market. At least two quarter of kilogram unique samples were coalesced from each trader for laboratory analysis. Samples were collected after thoroughly mixing maize in the bag to increase chances of getting the fungi. The samples were stored at temperatures below 4° C to await analysis.

3. Detection of aflatoxins and fumonisins by ELISA method

3.1 Procedure of sample preparation

A representative sample was taken and it was grounded with blender so that 75 % of that grounded portion can pass through a 20-mesh sieve, then thoroughly the sub-sample portion was mixed. 50 g of ground sample was weighed out into a clean conical flask that can be tightly sealed. 250 mL of methanol (70 % methanol diluted in water) extraction solution was added to the ground sample and the flask was sealed. Then the conical flask containing the sample was shaken for 3 min. The sample was allowed to settle down, then the top layer of extract was filtered through a Whatman #1 filter paper and the filtrate sample was collected. The prepared extract was diluted at 1:20 with distilled water. Sample was ready for testing without further preparation.

3.2 Assay protocol for aflatoxins

200 μL conjugate solution was pipetted into dilution wells. 100 μL of each standard or sample extract was added into the dilution wells. The mixture was mixed well and 100 μL of the mixture (conjugate and standard or samples) was transferred into antibody-coated wells. The plate was then incubated for 15 min with slow shaking and washed with distilled water for 5 times. The plate was then tap dried. 100 μL of substrate solution was pipetted into antibody coated wells. The plate was incubated with shaking for 5 min. 100 μL of stop solution was pipetted into antibody coated wells. The absorbance of each well was read at 450 nm with a differential filter at 630 nm. As the aflatoxin limit was (0–40) ppb but we found more than that which was diluted by dilution factor in three regions (Bogura, Nilphamari, Rangpur) by four times dilution.

3.3 Assay procedure for fumonisins detection

200 μL conjugate solution was pipetted into dilution wells with 100 μL of each standard and sample extract. The mixture was mixed well and 100 μL of the mixture (conjugate and standard or samples) was transferred into antibody-coated wells. The plate was then incubated for 15 min with slow shaking and then washed with distilled water for 5 times. The plate was then tap dried. 100 μL of substrate solution was pipetted into antibody coated wells. The plate was incubated with shaking for 5 min. 100 μL of stop solution was pipetted into antibody coated wells. The absorbance of each well was read at 450 nm with a differential filter at 630 nm.

3.4 Isolation, purification, identification and preservation of mycotoxigenic fungi

Isolation & purification of Aspergillus spp. and Fusarium spp. were collected from stored maize grain samples which was conducted by blotter method [64, 65]. In this
method, 400 maize grains were tested for the identification of toxigenic Aspergillus spp. and Fusarium spp. for each sample collected from different locations and 40 plastic petridishes were used for each sample. Then 10 maize grains were placed in the sterile plastic petridish containing three layers of wet blotter papers. The petridish was incubated at 25 ± 1°C under 12/12 h light and darkness cycle for 7 days. Each seed was observed under stereo microscope (Stemi 508, Germany) in order to record the presence of fungal colonies and temporary slides were prepared from the fungal colonies for morphological identification under compound microscope (Primo Star, Germany). Or one of the quarter kilo samples from each trader milled into fine flour using a Laboratory Milling machine. Ten grams of the ground sample was mixed with 100 ml sterile water to make a stock solution and serially diluted up to dilution 10^3. The suspension was plated in Potato Dextrose Agar Medium (PDA) [66, 67] by mixing 1 ml suspension in molten PDA cooled to 40°C. Isolation media was prepared by weighing 39 g of PDA into 1 L of water. The mixture was autoclaved for 15 min at 121°C and 15 PSI pressure. The media was allowed to cool to about 50°C and then amended with 25 ng/L of streptomycin and tetracycline [68, 69]. Petri dishes were labeled appropriately and a milliliter of the diluted sample was poured into a sterile petri dish aseptically and then 18 ml of PDA media at 40°C will was poured on the same plate and the mixture swirled gently to mix. The mixture was allowed to cool and solidify in the laminar flow hood and then sealed using parafilm for incubation. The plates were incubated at room temperature for 5–7 days.

4. Molecular based identification of fungi

4.1 DNA extraction

Before DNA extraction each purified Aspergillus spp. and Fusarium spp. was grown on PDA for 7–10 days at 28°C in an incubator. Then a 5 mm culture block was transferred on the conical flask containing PDA broth and the flasks were incubated at 28°C in an incubator for 7–10 days. Mycelium of each isolate was harvested and preserved at −80°C.

Genomic DNA was extracted from the fungal species isolated from maize grains following Wizard Genomic DNA extraction kit (Promega, USA) according to the manufacturer instructions from 100 mg fungal tissue ground with liquid nitrogen. Fungal tissue was processed by freezing with liquid nitrogen and grinding into a fine powder using a microcentrifuge tube pestle or a mortar and it was pestled. 0.04 g of this fungal tissues powder was added to a 1.5 ml microcentrifuge tube. 600 μl of Nuclei Lysis Solution was added and it was vortexed for 1–3 s to wet the tissue. The sample was incubated at 65°C for 15 min. 3 μl of RNase Solution was added to the cell lysate, and the sample was mixed by inverting the tube 2–5 times. The mixture was incubated at 37°C for 15 min. The sample was allowed to cool to room temperature for 5 min before proceeding. 200 μl of Protein Precipitation Solution was added, and it was vortexed vigorously at high speed for 20 s. The sample was centrifuged for 3 min at 13,000–16,000 × g. The precipitated proteins were formed into a tight pellet. The supernatant was carefully removed containing the DNA (leaving the protein pellet behind) and it was transferred to a clean 1.5 ml microcentrifuge tube containing 600 μl of room temperature isopropanol. The solution was gently mixed by inversion until thread-like strands of DNA form a visible mass. Then the sample was centrifuged at 13,000–16,000 × g for 1 min at room temperature. The supernatant carefully decanted. 600 μl of room temperature 70% ethanol was added and was inverted gently into the tube several times to wash the DNA. It was centrifuged at 13,000–16,000 × g for 1 min at room temperature. The
ethanol was aspirated carefully using either a drawn Pasteur pipette or a sequenc-
ing pipette tip. The DNA pellet was very loose at this point and care must be used to avoid aspirating the pellet into the pipette. The tube was inverted onto clean absorbent paper and the pellet was air-dried for 15 min. 100 μl of DNA Rehydration Solution was added and the DNA was rehydrated by incubating at 65° C for 1 h. Periodically the solution was mixed by gently tapping the tube. Alternatively, the DNA was rehydrated by incubating the solution overnight at room temperature or at 4° C. The DNA was stored at 2–8° C.

4.2 Primers, PCR conditions and sequencing of ITS region

The extracted DNA samples were amplified with PCR reaction for ITS regions. The forward primer: ITS1-5.8S (5′-GGAAGTAAAAGTCTGATAAAC-3′) and the reverse primer rDNA-ITS4 (TCCCTCCGCTATTGATATGC) were used [70]. PCRs were performed in 25 μl reaction volume containing 12.5 μl master mix, 1 μl ITS1, 1 μ ITS4, 9.5 μl Nuclease free water and 1 μl templet DNA (100 ng/μl). PCR products were visualized in 2% agarose gel, dyed with ethidium bromide and the photograph was taken using a Gel documentation system (Dynamica, GelView Master). The conditions for PCR reaction was: initial denaturation for 5 min at 95° C, followed by 34 cycles at 95° C for 30s, at 55° C for 1 min and at 72° C for 1 min and then final elongation at 72° C for 6 min. The amplified products were stored at −20° C. PCR products were sequenced using ITS1 primer via commercial outsourcing at Macrogen, Korea via Biotech concern. Finally, Sequence data were imported by Chromas Software version 2. Sequence data were analyzed by BLAST program (Basic Local Alignment Search Tool) and GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

5. PCR based detection of aflatoxin producing Aspergillus spp

5.1 PCR primers and amplification

Primers nor-1 FP (5′-ACCGCTACGCGCCGACTCTCGGCAC-3′) and nor-1 RP (5′-GTTGGCCGCCAGCTTCGACTCCTCG-3′) were set to amplify an amplicon of 400 bp of norsolorinic acid reductase; omtA FP (5′-GGCCCGTTCTCCCTG GCTCCTAAGC-3′) and omtA RP (5′-CGCCCCAGTGAGACCCTTCC TCG-3′) to amplify a 1024 bp fragment of sterigmatocystin O-methyltransferase; and aflR FP (5′-TATCTCCCCCGGGCATCTCCCCG-3′) and aflR RP (5′-CCGTCAGACGCAACTGGACACG-3′) to amplify a 1032 bp fragment of regulatory protein (aflR), involved in aflatoxin biosynthesis. The nucleotide sequence of all these genes from A. parasiticus are available at NCBI, GenBank at accession numbers L27801 (nor-1), SRR 2043 (aflR) and SRR 143 (omt-1). PCR was performed in 15 μL of reaction volume containing 7.5 μl master mix, 1 μl forward primer, 1 μl of reverse primer and 4.5 μl nuclease free water and 1 μl of extracted DNA as template (with a total concentration of 100 ng of genomic DNA per reaction). PCR condition for nor 1 primer initial denaturation for 5 min at 94° C, followed by 35 cycles at 94° C for 30 s, at 67° C for 30 s and at 72° C for 30 s and then final elongation at 72° C for 10 min [71]. PCR condition for omtA and aflR primer initial denaturation for 10 min at 95° C, followed by 30 cycles at 94° C for 1 min, at 65° C for 2 min and at 72° C for 2 min and then final elongation at 72° C for 5 min [71]. PCR products were separated by electrophoresis on a 1% agarose gel with 0.5% ethidium bromide in 1× TBE buffer and visualized under a Gel document-
tion system (Dynamica, GelView Master). 1 kb plus DNA Ladder (BioLabs, New England) was used as molecular size marker for the analysis of fragment size.
6. PCR based identification of mycotoxigenic *Fusarium* spp

6.1 Primers for PCR amplification

Primers specific for fumonisins producing *Fusarium* spp. (*FUM1* Forward-CCATCAC AGTGGGACACAGT, *FUM1* Reverse-CGTATC GTCAGCATGATGTAGC) were used previously [72]. PCR were performed in mixture 15 μl volume containing 1 μl of DNA sample, 7.5 μl of master mix, 1 μl *FUM1* forward primer, 1 μl *FUM1* reverse primer, 4.5 μl nuclease free water. PCR was performed using T100 Thermocycler (BioRad, Hercules, USA). The PCR condition for *FUM1* regions include 94°C for 4 min for initial denaturation, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min, primer extension at 72°C for 1 min. The final extension was set at 72°C for 10 min. 4 μl of the PCR product was electrophoresed on 1.5 % agarose gel, stained with ethidium bromide, illuminated and documented using Gel documentation system (Dynamica, GelView Master).

7. Statistical analyses

The collected data were analyzed statistically by using Minitab software version 17 (www.minitab.com). The mean of all the treatments were compared by critical difference value at 5 % level of significance.

8. Results

8.1 Determination of total Aflatoxins contamination in stored maize grain samples collected from some selected growing areas of Bangladesh

The study was performed at the Laboratory of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Composite stored maize grain samples were collected from 15 maize growing areas of Bangladesh including Panchagarh, Thakurgaon, Dinajpur, Nilphamari, Rangpur, Lalmonirhat, Gaibandha, Bogura, Natore, Kushtia, Jashore, Chuadanga, Kishoreganj, Manikganj and Cumilla.

In terms of total aflatoxins concentration in μg/kg, the highest and lowest amount of aflatoxins concentration was recorded in Chuadanga (101.57 μg/kg) and Dinajpur (1.08 μg/kg) which exposed no significant relationship to each other. The moderate amount of aflatoxin level was detected in Gaibandha (68.73 μg/kg), Kushtia (31.48 μg/kg), Kishoreganj (30.86 μg/kg), Rangpur (20.56 μg/kg) and Cumilla (11.91 μg/kg) revealing more aflatoxins contamination than the regulatory limit (10 μg/kg) in which only aflatoxins concentration from Kushtia and Kishoreganj revealed statistically significant data, besides, rest of the location exhibited below level of aflatoxins contamination of regulatory limit showing more or less statistically significant data.

Total aflatoxins associated with maize grains were detected in 2020, with the supreme value was detected in Chuadanga (30.5 %) followed by Kushtia (29.5 %), Nilphamari (22.5 %), Panchagarh (19.25 %) and the minimal aflatoxins was detected in Manikganj (3.2 %), rest of the samples from other districts revealed lower to moderate level of aflatoxins, moreover, data from Chuadanga and Kushtia, Cumilla, Jashore and Natore, Thakurgaon and Rangpur, Lalmonirhat and Kishoreganj regions revealed ststistically similar data while data from other regions exhibited statistically dissimilar data.

In case of infection rate, toxgenic maize samples were obtained from Panchagarh, Thakurgoan, Gaibandha, Chuadanga, Kishoreganj exhibiting 100 % infection by
A. flavus and no atoxigenic samples were found in those area. Moderate amount of toxigenic A. flavus was detected in Jashore followed by Cumilla, Natore, Lalmonirhat, Nilphamari which were 78 %, 75 %, 66 %, 50 % respectively and atoxigenic fungi was detected 22 %, 25 %, 34 %, 50 %, 50 % were detected respectively. Rest of the locations (Dinajpur, Rangpur, Bogura, Kushtia, Manikganj) exhibited higher amount of atoxigenic A. flavus compared to toxigenic A. flavus (Table 1).

The outmost percent aflatoxins concentration over standard limit was found in Chuadanga (915.7 %) followed by Gaibandha (587.3 %), Kushtia (214.8 %), Kishoreganj (208.85 %), Rangpur (105.6 %), Cumilla (19.5 %) revealing that the aflatoxins contamination from those area were beyond the regulatory limit set by EU for aflatoxins (10 ppb), conversely, aflatoxin concentration from other nine locations were below the regulatory limit of aflatoxins (Table 1).

8.2 Relationship between aflatoxins producing A. flavus and mean aflatoxins concentrations

The regression analysis between toxigenic A. flavus percentage and mean aflatoxin concentrations which was positively correlated by observing regression equation where the slope was = 0.55 and y-intercept was = 50.14, coefficient of

| Location          | Total aflatoxins concentrations (μg/kg) | % A. flavus associated with maize grains | Percent total aflatoxins concentration over standard limit |
|-------------------|----------------------------------------|----------------------------------------|----------------------------------------------------------|
|                   | Total | Toxigenic | Atoxigenic   |                                                             |
| Panchagarh        | 4.96 ± 0.19f | 19.25 ± 3.53e | 100 | 0 | — |
| Thakurgoan        | 1.28 ± 0.10f | 18.25 ± 0.43d | 100 | 0 | — |
| Dinajpur          | 1.08 ± 0.12f | 16 ± 1.73de | 25 | 75 | — |
| Nilphamari        | 3.04 ± 0.56f | 22.5 ± 3.28b | 50 | 50 | — |
| Rangpur           | 20.56 ± 0.42f | 18.5 ± 2.18ed | 44 | 56 | 105.6 |
| Lalmonirhat       | 3.37 ± 0.19f | 9.75 ± 1.00f | 50 | 50 | — |
| Gaibandha         | 68.73 ± 4.02f | 3.75 ± 1.00h | 100 | 0 | 5873 |
| Bogura            | 3.33 ± 0.41f | 11.25 ± 0.66f | 40 | 60 | — |
| Natore            | 2.39 ± 1.29f | 13.5 ± 1.80ed | 66 | 34 | — |
| Kushtia           | 31.48 ± 1.14c | 29.5 ± 1.32c | 33 | 67 | 214.8 |
| Jashore           | 1.67 ± 0.57f | 13.75 ± 1.64ef | 78 | 22 | — |
| Chuadanga         | 101.57 ± 5.09f | 30.5 ± 0.50f | 100 | 0 | 915.7 |
| Kishoreganj       | 30.89 ± 0.22c | 10.25 ± 1.09ef | 100 | 0 | 208.85 |
| Manikganj         | 2.57 ± 0.01f | 3.25 ± 0.43b | 33.33 | 66.67 | — |
| Cumilla           | 11.91 ± 0.30f | 14 ± 2.00ef | 75 | 25 | 19.5 |

*Significant at 5 % level of significance. Least significant difference (LSD) at P = 0.05 was used for comparing means and the P values were 0.00.

**Significant at 1 % level of significance. Least significant difference (LSD) at P = 0.05 was used for comparing means and the P values were 0.00. Data are the averages of three biological replications. The regulatory limits for fumonisin is 1 ppm (10 μg/kg).

Table 1.
Levels of Total aflatoxins concentration in stored maize grains collected from the stores of traders of fifteen maize growing areas of Bangladesh.
determination, $R^2 = 0.198$ and coefficient of correlation, $r = 0.44$ which depicted that 1% surges of toxigenic *A. flavus* in maize grains ultimately rised 50.137 μg/kg aflatoxin concentration. In terms of 5% surges of toxigenic *A. flavus* in maize grains, the aflatoxin concentration was increased up to 2.75 μg/kg and when toxigenic *A. flavus* increased 20% in maize grains, the aflatoxin concentration was escalated up to 11.0 μg/kg (Figure 1).

8.3 Identification of *A. flavus* from the stored maize grain samples collected from some selected growing areas of Bangladesh

Morphological identification of *A. flavus* was detected by using petridish and culture plate method as well as observing microscopic figures under compound and stereo microscope (Figure 2A(a)–(d)). Thirty five fungal isolates were identified using primers specific to ITS 1 and ITS 4 regions. PCR assays of *A. flavus* DNA with ITS 1 and ITS 4 primers amplified a single fragment of about 600 bp which revealed that all the isolates obtained were fungi. Sequence analysis of ITS region by BLAST program revealed that all the isolates obtained from maize were belong to *A. flavus* (Figure 3A).

8.4 PCR based identification and confirmation of aflatoxin producing *Aspergillus flavus* species obtained from maize grain samples

AF02_Ran, AF01_Lal, AF01_Bog, AF02_Bog, AF03_Jas, AF04_Jas, AF01_Chu, AF03_Kis, AF04_Kis, AF01_Man were identified by PCR amplification of ITS region using ITS1 and ITS4 primers and the results of PCR showed an amplification size 600 bp confirmed the fungal isolates (Figure 3A) and their several strains were found in Rangpur (*A. flavus* strain 64-A1), Lalmonirhat (*A. flavus* strain SGE22), Bogura (*A. flavus* strain SGE22 and *A. flavus* strain bpo4), Jashore (*A. flavus* and *A. flavus* isolate AA22), Chuadanga (*A. flavus* strain JN-YG-3-5), Kishoreganj (*A. flavus* strain 64-A1 and *A. flavus* strain ND26), Manikganj (*A. flavus* strain SU-16).

PCR products were then sequenced and analysis of sequence data of amplified ITS region using BLAST program revealed that fungal isolates AF01_Man, AF03_Jas, AF02_Ran obtained from maize grain samples collected from Manikganj, Jashore, Rangpur revealed the highest homology of 99.33 %, 99.17 %, 95.74 % with the *A. flavus* strain SU-16, *A. flavus*, *A. flavus* strain 64-A1. Other sevel isolates obtained from Lalmonirhat (AF01_Lal), Bogura (AF01_Bog), Bogura (AF02_Bog), Jashore (AF04_Jes), Chuadanga (AF01_Chu), Kishoreganj (AF03_Kis), Kishoreganj (AF04_Kis) showed significant homology with different strains of *A. flavus* (Table 2).
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Figure 2.
(A) Composite photographs of Aspergillus spp. in different sections. (a) Apparent growth of Aspergillus spp. on the maize grain surface, (b) enlarged view of individual maize grain showing the growth of Aspergillus spp., morphology of suspected Aspergillus spp. (c) Yellowish green colonies of A. flavus on PDA, (d) vesicle with less conidial ornamentation with conidiphores of A. flavus. (B) Composite photographs of Fusarium spp. in different sections. (a) Apparent growth of Fusarium spp. on the maize grain surface, (b) enlarged view of individual maize grain showing the growth of Fusarium spp., morphology of suspected Fusarium spp., (c) pinkish white growth of F. proliferatum on PDA, (d) microconidia of F. proliferatum without septum under microscope with 40X magnification, (e) whitish growth of F. oxysporum on PDA and (f) Micro and macroconidia (with septum) of F. oxysporum without septum. Culture photographs were taken at 7 days after inoculation and microscopic photographs were taken with 40X magnification using compound light microscope equipped with a digital camera.

Figure 3.
(A) PCR amplification of ITS region from the genomic DNA of the fungal isolates using ITS-1 and ITS-4 primers and (B) PCR amplification of nor, omr, apa-2 gene from the genomic DNA of the fungal isolates obtained from fifteen maize growing areas of Bangladesh M: 1 kb plus DNA ladder, 1, AF02_Ran: Rangpur, 2, AF01_Lal: Lalmonirhat, 3, AF01_Bog: Bogura, 4, AF02_Bog: Bogura, 5, AF03_Jas: Jassore, 6, AF04_Jas: Jashore, 7, AF03_Chu: Chuadanga, 8, AF03_Kis: Kishoreganj, 9, AF04_Kis: Kishoreganj, 10, AF01_Man: Manikgan.
| Isolate ID | Location  | Identity | Accession numbers | Clones closest relatives | Aflatoxins biosynthesis genes | Comment |
|------------|-----------|----------|-------------------|---------------------------|------------------------------|---------|
| AF01_Pan   | Panchagarh | A. flavus isolate PA23 | MN006654.1, AF01_Pan | A. flavus isolate PA23 | +                       |          |
| AF02_Pan   | Panchagarh | A. flavus isolate AF15 | KX253943.1, AF02_Pan | A. flavus isolate AF15 | +                       |          |
| AF01_Tha   | Thakurgaon | A. flavus isolate AA221 | MN006401.1, AF01_Tha | A. flavus isolate AA221 | +                       |          |
| AF02_Tha   | Thakurgaon | A. flavus isolate Z15 | MT537941.1, AF02_Tha | A. flavus isolate Z15 | +                       |          |
| AF01_Din   | Dinajpur   | A. flavus isolate 20-117 | MT537941.1, AF01_Din | A. flavus isolate 20-117 | +                       |          |
| AF02_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF02_Din | A. flavus isolate SCF34 | +                       |          |
| AF01_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF01_Bog | A. flavus strain isolate A3 | +                       |          |
| AF02_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF02_Bog | A. flavus strain isolate A3 | +                       |          |
| AF01_Nat   | Natore     | A. flavus strain BLND-1 | MT5946351.1, AF01_Nat | A. flavus strain BLND-1 | +                       |          |
| AF02_Nat   | Natore     | A. flavus strain GFRS16 | MT5946351.1, AF02_Nat | A. flavus strain GFRS16 | +                       |          |
| AF01_Nil   | Nilphamari | A. flavus isolate AA221 | MT537941.1, AF01_Nil | A. flavus isolate AA221 | +                       |          |
| AF02_Nil   | Nilphamari | A. flavus isolate 20-117 | MT537941.1, AF02_Nil | A. flavus isolate 20-117 | +                       |          |
| AF01_Tha   | Thakurgaon | A. flavus isolate 20-117 | MT537941.1, AF01_Tha | A. flavus isolate 20-117 | +                       |          |
| AF02_Tha   | Thakurgaon | A. flavus isolate SCF34 | MT537941.1, AF02_Tha | A. flavus isolate SCF34 | +                       |          |
| AF01_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF01_Din | A. flavus isolate SCF34 | +                       |          |
| AF02_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF02_Din | A. flavus isolate SCF34 | +                       |          |
| AF01_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF01_Bog | A. flavus strain isolate A3 | +                       |          |
| AF02_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF02_Bog | A. flavus strain isolate A3 | +                       |          |
| AF01_Nat   | Natore     | A. flavus strain BLND-1 | MT5946351.1, AF01_Nat | A. flavus strain BLND-1 | +                       |          |
| AF02_Nat   | Natore     | A. flavus strain GFRS16 | MT5946351.1, AF02_Nat | A. flavus strain GFRS16 | +                       |          |
| AF01_Nil   | Nilphamari | A. flavus isolate AA221 | MT537941.1, AF01_Nil | A. flavus isolate AA221 | +                       |          |
| AF02_Nil   | Nilphamari | A. flavus isolate 20-117 | MT537941.1, AF02_Nil | A. flavus isolate 20-117 | +                       |          |
| AF01_Tha   | Thakurgaon | A. flavus isolate 20-117 | MT537941.1, AF01_Tha | A. flavus isolate 20-117 | +                       |          |
| AF02_Tha   | Thakurgaon | A. flavus isolate SCF34 | MT537941.1, AF02_Tha | A. flavus isolate SCF34 | +                       |          |
| AF01_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF01_Din | A. flavus isolate SCF34 | +                       |          |
| AF02_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF02_Din | A. flavus isolate SCF34 | +                       |          |
| AF01_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF01_Bog | A. flavus strain isolate A3 | +                       |          |
| AF02_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF02_Bog | A. flavus strain isolate A3 | +                       |          |
| AF01_Nat   | Natore     | A. flavus strain BLND-1 | MT5946351.1, AF01_Nat | A. flavus strain BLND-1 | +                       |          |
| AF02_Nat   | Natore     | A. flavus strain GFRS16 | MT5946351.1, AF02_Nat | A. flavus strain GFRS16 | +                       |          |
| AF01_Nil   | Nilphamari | A. flavus isolate AA221 | MT537941.1, AF01_Nil | A. flavus isolate AA221 | +                       |          |
| AF02_Nil   | Nilphamari | A. flavus isolate 20-117 | MT537941.1, AF02_Nil | A. flavus isolate 20-117 | +                       |          |
| AF01_Tha   | Thakurgaon | A. flavus isolate 20-117 | MT537941.1, AF01_Tha | A. flavus isolate 20-117 | +                       |          |
| AF02_Tha   | Thakurgaon | A. flavus isolate SCF34 | MT537941.1, AF02_Tha | A. flavus isolate SCF34 | +                       |          |
| AF01_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF01_Din | A. flavus isolate SCF34 | +                       |          |
| AF02_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF02_Din | A. flavus isolate SCF34 | +                       |          |
| AF01_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF01_Bog | A. flavus strain isolate A3 | +                       |          |
| AF02_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF02_Bog | A. flavus strain isolate A3 | +                       |          |
| AF01_Nat   | Natore     | A. flavus strain BLND-1 | MT5946351.1, AF01_Nat | A. flavus strain BLND-1 | +                       |          |
| AF02_Nat   | Natore     | A. flavus strain GFRS16 | MT5946351.1, AF02_Nat | A. flavus strain GFRS16 | +                       |          |
| AF01_Nil   | Nilphamari | A. flavus isolate AA221 | MT537941.1, AF01_Nil | A. flavus isolate AA221 | +                       |          |
| AF02_Nil   | Nilphamari | A. flavus isolate 20-117 | MT537941.1, AF02_Nil | A. flavus isolate 20-117 | +                       |          |
| AF01_Tha   | Thakurgaon | A. flavus isolate 20-117 | MT537941.1, AF01_Tha | A. flavus isolate 20-117 | +                       |          |
| AF02_Tha   | Thakurgaon | A. flavus isolate SCF34 | MT537941.1, AF02_Tha | A. flavus isolate SCF34 | +                       |          |
| AF01_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF01_Din | A. flavus isolate SCF34 | +                       |          |
| AF02_Din   | Dinajpur   | A. flavus isolate SCF34 | MT537941.1, AF02_Din | A. flavus isolate SCF34 | +                       |          |
| AF01_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF01_Bog | A. flavus strain isolate A3 | +                       |          |
| AF02_Bog   | Bogura     | A. flavus strain isolate A3 | MT80401.1, AF02_Bog | A. flavus strain isolate A3 | +                       |          |
| AF01_Nat   | Natore     | A. flavus strain BLND-1 | MT5946351.1, AF01_Nat | A. flavus strain BLND-1 | +                       |          |
| AF02_Nat   | Natore     | A. flavus strain GFRS16 | MT5946351.1, AF02_Nat | A. flavus strain GFRS16 | +                       |          |
| Isolate ID  | Location   | Closest relatives          | Accession numbers | Identity | Homology (%) | Aflatoxins biosynthesis genes | Comment |
|------------|------------|----------------------------|-------------------|----------|--------------|-----------------------------|---------|
| AF04_Jas   | Jashore    | *A. flavus* isolate AA221 | MN006401.1        | 229/241  | 95           | +                           | +       |
| AF05_Jas   | Jashore    | *A. flavus* strain BLND1-1 | MN396712.1        | 157/164  | 95.73        | +                           | —       |
| AF06_Jas   | Jashore    | *A. flavus* strain A1     | CP051065.1        | 551/587  | 93.87        | +                           | —       |
| AF07_Chu   | Jashore    | *A. flavus* strain FG38   | EU30347.1         | 38/39    | 97.44        | +                           | —       |
| AF01_Chu   | Chuadanga  | *A. flavus* strain JNYG-3-5 | MG554231.1      | 413/457  | 90.37        | +                           | +       |

Table 2.
List of *A. flavus* isolates identified by homology search of sequences of ITS region by BLAST program obtained from maize grain samples collected from fifteen growing areas of Bangladesh.
When the isolates of *Aspergillus* Spp. were analyzed by PCR for aflatoxin producing ability using *nor*, *omtA*, *apa-2* genes based primers from fifteen maize growing areas. The result showed the amplified DNA fragment was 400 bp, 1024 bp, 1032 bp confirmed that the *A. flavus* isolates had the ability to produce aflatoxin that encode *nor*, *omtA*, *apa-2* genes (Figure 3B). Only six species showed a positive result with *nor*, *omtA*, *apa-2* genes set of primers. The result indicated *A. flavus* strains were aflatoxins producers as those were an evident from our investigation (Figure 3B).

PCR products were sequenced using ITS-1 primer and sequence data were analyzed by homology search using BLAST Nucleotide program. Isolates were identified as different *A. flavus* based on the homology percentage with their closest relatives available in the NCBI database.

9. Determination of total fumonisins contamination in stored maize grain samples collected from some selected growing areas of Bangladesh

The study was conducted at the Laboratory of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Composite stored maize grains samples were collected from 15 maize growing areas of Bangladesh such as Panchagarh, Thakurgaon, Dinajpur, Nilphamari, Rangpur, Lalmonirhat, Gaibandha, Bogura, Natore, Kushtia, Jashore, Chuadanga, Kishoreganj, Manikganj, Cumilla.

Fumonisins were detected with the highest value recorded in Gaibandha (9.18 mg/kg) and the lowest in Cumilla (0.11 mg/kg) (Table 3). Panchagarh (1.47 mg/kg), Thakurgaon (1.27 mg/kg), Dinajpur (0.65 mg/kg), Nilphamari (1.28 mg/kg), Rangpur (1.65 mg/kg), Lalmonirhat (1.18 mg/kg), Bogura (1.29 mg/kg), Kushtia (1.44 mg/kg), Kishoreganj (1.54 mg/kg), and Manikganj (1.47 mg/kg) had moderately high fumonisin levels revealing statistically identical data. Other regions showed indentically dissimilar data except Natore (0.23 mg/kg) and Chuadanga (0.59 mg/kg) (Table 3).

Infection rate of *Fusarium* spp. had the highest value in Bogura (13.50 %) followed by Gaibandha (13.25 %), Nilphamari (12.50 %) depicted statistically similar data and the minimal was found in Chuadanga (0.50 %) and Kustia (0.56 %). Moderately higher levels of fumonisin detected in Panchagarh (2.63 %), Thakurgaon (6.06 %), Dinajpur (2.38 %), Rangpur (9.69 %), Jessore (2.25 %), Kishoreganj (17.88 %), Manikganj (6.94 %) and Cumilla (5.31 %) were in the group of statistically identical data. Moderate but less high and statistically similar results showed in Thakurgaon (6.06 %) and Manikganj (6.94 %) (Table 3).

The outmost percent fumonisins concentration over standard limit was found in Rangpur (65 %) followed by Kishoreganj (53.5 %), Gaibandha (47.5 %), Manikganj (47 %), Kushtia (45 %), Panchagarh (46.5 %), Bogura (28.5 %), Thakurgaon (27 %), Nilphamari (27 %), Lalmonirhat (18 %) revealing that the aflatoxins contamination from those area were beyond the regulatory limit set by EU for fumonisins (1 ppm), conversely, fumonisins concentration from other five locations were below the regulatory limit of fumonisins (1 ppm) (Table 3).

9.1 Relationship between fumonisins producing *Fusarium* spp. and mean fumonisin concentrations

The regression analysis between *Fusarium* spp. infected maize grains and mean fumonisin concentrations which was positively correlated by observing regression equation where the slope was = 0.038 and y-intercept was = 0.882, coefficient of
determination, $R^2 = 0.198$ and coefficient of correlation, $r = 0.45$ which depicted that 1 percent surges of *Fusarium* in maize grains ultimately rised 0.038 mg/kg fumonisins concentration. In terms of 5% surges of *Fusarium* in maize grains, the fumonisins concentration was increased up to 0.19 mg/kg and when *Fusarium* increased 20% in maize grains, the fumonisins concentration was escalated up to 0.76 mg/kg (Figure 4).

| Location     | Total fumonisins (mg/kg) | Percent maize grains infected with *Fusarium* species | Percent total Fumonisins concentration over standard limit |
|--------------|--------------------------|------------------------------------------------------|----------------------------------------------------------|
| Panchagarh   | 1.47 ± 0.14$^b$          | 2.63 ± 1.20$^c$                                      | 46.5                                                     |
| Thakurgoan   | 1.27 ± 0.13$^d$          | 6.06 ± 2.07$^d$                                      | 27                                                       |
| Dinajpur     | 0.65 ± 0.01$^d$          | 2.38 ± 0.54$^d$                                      | —                                                        |
| Nilhamari    | 1.28 ± 0.11$^b$          | 12.50 ± 0.89$^b$                                     | 27                                                       |
| Rangpur      | 1.65 ± 0.27$^d$          | 9.69 ± 2.33$^b$                                      | 65                                                       |
| Lalmoinirhat | 1.18 ± 0.17$^bc$         | 0.00 ± 0.00$^b$                                      | 18                                                       |
| Gaibandha    | 9.18 ± 1.02$^c$          | 13.25 ± 1.39$^c$                                     | 47.5                                                     |
| Bogura       | 1.28 ± 0.33$^b$          | 13.50 ± 1.5$^b$                                      | 28.5                                                     |
| Natore       | 0.23 ± 0.06$^b$          | 0.00 ± 0.00$^b$                                      | —                                                        |
| Kushitia     | 1.44 ± 0.1$^b$           | 0.56 ± 0.41$^b$                                      | 45                                                       |
| Jashore      | 0.75 ± 0.10$^d$          | 2.25 ± 0.43$^d$                                      | —                                                        |
| Chuaadanga   | 0.59 ± 0.07$^b$          | 0.50 ± 0.50$^b$                                      | —                                                        |
| Kishoreganj  | 1.54 ± 0.2$^b$           | 7.88 ± 0.92$^b$                                      | 53.5                                                     |
| Manikganj    | 1.47 ± 0.2$^b$           | 6.94 ± 0.91$^d$                                      | 47                                                       |
| Cumilla      | 0.11 ± 0.01$^e$          | 5.31 ± 0.35$^e$                                      | —                                                        |

**Level of significance**
- **Significant at 5% level of significance. Least significant difference (LSD) at $P = 0.05$ was used for comparing means and the P values were 0.00.**
- **Significant at 1% level of significance. Least significant difference (LSD) at $P = 0.05$ was used for comparing means and the P values were 0.00. Data are the averages of three biological replications. The regulatory limits for fumonisin is 1 ppm (1 mg/kg).**

Table 3.
Levels of total fumonisins concentration in stored maize grains collected from the stores of traders of fifteen maize growing areas of Bangladesh.

determination, $R^2 = 0.198$ and coefficient of correlation, $r = 0.45$ which depicted that 1 percent surges of *Fusarium* in maize grains ultimately rised 0.038 mg/kg fumonisins concentration. In terms of 5% surges of *Fusarium* in maize grains, the fumonisins concentration was increased up to 0.19 mg/kg and when *Fusarium* increased 20% in maize grains, the fumonisins concentration was escalated up to 0.76 mg/kg (Figure 4).

9.2 Identification of *Fusarium* species from the stored maize grain samples collected from some selected growing areas of Bangladesh

Morphological identification of *F. oxysporum* and *F. proliferatum* were detected by using petridish and culture plate method as well as observing microscopic figures under compound and stereo microscope (Figure 2B(a)-(f)). Fifteen fungal isolates were identified using primers specific to ITS 1 and ITS 4 region. PCR assays of *F. oxysporum* DNA with ITS 1 and ITS 4 primers amplified a single fragment of about 600 bp which revealed that all the isolates obtained were fungi (Figure 5A). Sequence analysis of ITS region by BLAST program revealed that all the isolates obtained from maize were belong to *F. oxysporum* and *F. proliferatum*. 
10. PCR based identification and confirmation of fumonisins producing *Fusarium* species obtained from maize grain samples

F01_Pan, F02_Tha, F03_Din, F04_Nil, F05_Ran, F06_Lal, F07_Gai, F08_Bog, F09_Nat, F010_Kus, F011_Jes, F012_Chu, F013_Kis, F014_Man and F015_Cum were identified by PCR amplification of ITS region using ITS1 and ITS4 primers and the results of PCR showed an amplification size 600 bp confirmed the *Fusarium*. PCR products were then sequenced. (Figure 2A). Out of fifteen maize growing areas, *F. oxysporum* was found in Panchagarh (*F. oxysporum* strain EP19), Thakurgaon (*F. oxysporum* strain En3), Dinajpur (*F. oxysporum* strain EP19), Nilphamari (*F. oxysporum* strain EP19), Rangpur (*F. oxysporum* strain En3), Natore

![Figure 4.](image)

Linear correlations between *Fusarium* infected maize grains and total fumonisin concentration.

![Figure 5.](image)

A. PCR amplification of ITS region from the genomic DNA of the fungal isolates using ITS-1 and ITS-4 primers and B. PCR amplification of FUM1 gene from the genomic DNA of the fungal isolates obtained from fifteen maize growing areas of Bangladesh M: 1 kb plus DNA ladder, 1, F01_Pan: Panchagarh, 2, F02_Tha: Thakurgaon, 3, F03_Din: Dinajpur, 4, F04_Nil: Nilphamari, 5, F05_Ran: Rangpur, 6, F06_Lal: Lalmonirhat, 7, F07_Gai: Gaibandha, 8, F08_Bog: Bogura, 9, F09_Nat: Natore, 10, F010_Kus: Kushtia, 11, F011_Jes: Jashore, 12, F012_Chu: Chuadanga, 13, F013_Kis: Kishoreganj, 14, F014_Man: Manikganj and 15, F015_Cum: Cumilla.
(F. oxysporum isolate FH10 18S), Kushtia (F. oxysporum strain EP19), Jashore (F. oxysporum strain En3), Chuadanga (F. oxysporum isolate H200714-017) Manikganj (F. oxysporum strain EP19), Cumilla (F. oxysporum strain En3) and F. proliferatum was found in Lalmonirhat (F. proliferatum strain TH11-3), Gaibandha (F. proliferatum strain TH11-3), Bogura (F. proliferatum strain TH11-3) and Kishoreganj (F. proliferatum strain TH11-3).

Fungal isolates F06_Lal, F07_Gai, F08_Bog and F013_Kis obtained from maize grain samples were collected from Lalmonirhat, Gaibandha, Bogura and Kishoreganj showed the highest homology with F. proliferatum strain TH11-3 (Table 4). The fungal isolates obtained from maize grain samples collected from

| Isolate ID | Location   | Closest relatives               | Accession number | Identity | Homology (%) |
|------------|------------|--------------------------------|------------------|----------|--------------|
| F01_Pan    | Panchagarh| F. oxysporum strain EP19       | MN704852.1       | 486/534  | 91.01        |
| F02_Tha    | Thakurgoan| F. oxysporum strain En3       | MN726603.1       | 491/537  | 91.43        |
| F03_Din    | Dinajpur  | F. oxysporum strain EP19       | MN704852.1       | 445/530  | 83.96        |
| F04_Nil    | Nilphamari| F. oxysporum strain EP19       | MN704852.1       | 486/534  | 91.01        |
| F05_Ran    | Rangpur   | F. oxysporum strain En3       | MN726603.1       | 477/539  | 88           |
| F06_Lal    | Lalmonirhat| Fusarium proliferatum strain TH11-3 | MT563411.1    | 472/508  | 92.91        |
| F07_Gai    | Gaibandha| Fusarium proliferatum strain TH11-3 | MT563411.1    | 472/508  | 92.91        |
| F08_Bog    | Bogura    | Fusarium proliferatum strain TH11-3 | MT563411.1    | 491/544  | 90           |
| F09_Nat    | Natore    | F. oxysporum isolate FH10 18S | KU361495.1      | 257/305  | 84.26        |
| F10_Kus    | Kushtia   | F. oxysporum strain EP19       | MN704852.1       | 486/534  | 91.01        |
| F011_Jes   | Jashore   | F. oxysporum strain En3       | MN726603.1       | 477/539  | 88           |
| F012_Chu   | Chuadanga| F. oxysporum isolate H200714-017 | MT974426.1    | 477/541  | 88.17        |
| F013_Kis   | Kishoreganj| F. oxysporum strain TH11-3   | MT563411.1      | 472/508  | 92.91        |
| F014_Man   | Manikganj| F. oxysporum strain EP19       | MN704852.1       | 486/534  | 91.01        |
| F015_Cum   | Cumilla   | F. oxysporum strain En3       | MN726603.1       | 477/539  | 88           |

PCR products were sequenced using ITS-1 primer and sequence data were analyzed by homology search using BLAST Nucleotide program. Isolates were identified as different Fusarium species based on the homology percentage with their closest relatives available in the NCBI database. F01_Pan: Panchagarh, F02_Tha: Thakurgoan, F03_Din: Dinajpur, F04_Nil: Nilphamari, F05_Ran: Rangpur, F06_Lal: Lalmonirhat, F07_Gai: Gaibandha, F08_Bog: Bogura, F09_Nat: Natore, F10_Kus: Kushtia, F011_Jes: Jashore, F012_Chu: Chuadanga, F013_Kis: Kishoreganj, F014_Man: Manikganj and F015_Cum: Cumilla.

Table 4.
List of Fusarium isolates identified by homology search of sequences of ITS region by BLAST program obtained from maize grain samples collected from fifteen growing areas of Bangladesh.
Panchagarh (F01_Pan), Thakurgaon (F02_Tha), Dinajpur (F03_Din), Nilphamari (F04_Nil), Rangpur (F05_Ran), Lalmonirhat (F06_Lal), Gaibandha (F07_Gai), Bogura (F08_Bog), Natore (F09_Nat), Kustia (F10_Kus), Jessore (F011_Jes), Chuadanga (F012_Chu), Kishoreganj (F013_Kis), Manikganj (F014_Man) and Cumilla (F015_Cum) showed significant homology with different strains of *F. oxysporum* (Table 4).

When the isolates of *Fusarium* species were analyzed by PCR for fumonisins producing ability using *FUM1* gene based primers from fifteen maize growing areas. The result showed the amplified DNA fragment was 183 bp confirmed that the *Fusarium* had the ability to produce fumonisin that encode *FUM1* gene (Figure 5B). Only two *Fusarium* species showed a positive result with *FUM1* gene set of primers. The result was contrary as *F. proliferatum* and *F. oxysporum* (Table 4) were fumonisin-producers as it was evident from our investigation.

11. Discussion

The experiment was conducted at Plant Bacteriology and Biotechnology Laboratory of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during the period of 2019–2020. The purpose of the experiment were to detect the levels of fumonisins and aflatoxins and to identify the aflatoxin and fumonisins producing *Aspergillus* and *Fusarium* in maize associated with maize by PCR using nor, omtA, apa-2 and *FUM1*. Genes involving *afl R*, *ver-1*, *omt-1* and *apa-2* associated with biosynthetic pathway regarding aflatoxins production [73–76]. *Apa-1, Nor-1, Omt-1* and *Ver-1* gens belong to four primers were applied to detect aflatoxins contamination [77, 78]. *A. flavus* was quantified by nor-1 gene in several contaminated food samples and cereals using PCR assay [77]. Besides, [56] mentioned that *FUM1* gene with an expected amplicon size of 183 bp can easily detect the fumonisin and non-fumonisin producing *Fusarium*, moreover other researchers also identified the fumonisin by using *FUM1* gene which is in accordance with our study [79–81]. We gathered samples from 15 maize growing areas to measure the aflatoxins and fumonisins level but not all the *Aspergillus* strains are capable of engendering mycotoxins, thus screening is crucial and we detected by Agra Quant Total Aflatoxin and Fumonisin Test Kit following ELISA approach for detection and this method also used by [82–87] for detecting aflatoxins and fumonisin. In our experiment, we detected the aflatoxins contamination Agra Quant Total Aflatoxins 96 well microtiter plate ELISA test kit produced in Romer Labs, Packers and Stockyards Administration (GIPSA) in US Department of Agriculture (USDA) which ability to detect individual aflatoxins very precisely and accurately with a range of 0–320 ppb in accrodance with an experiment conducted by [82]. A number of approaches have been widely used to detect mycotoxin naming high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and thin layer chromatography (TLC) [83, 84] and served as a reliable method for detecting aflatoxins and fumonisins [85, 88, 89]. In Gaibandha and Cumilla region fumonisin contamination were highest and lowest compared to other areas revealing moderate amount of fumonisins. In this study, all of the 15 samples were found positive with fumonisins producing *Fusarium* and aflatoxin producing fungi *Aspergillus* which in accordance with the findings of [90, 91]. We found positive correlation for both aflatoxins and fumonisins contamination between their toxin percentages which were matched with the findings of [92] who found apositive correlation has been identified between the proportion of *FUM1* transcripts and the proportion of fumonisins biosynthesized by the *F. verticillioides* and *F. proliferatum* species.
In case of Percent total Fumonisins concentration over standard limit, five regions were under the regulatory limit and other ten regions were exposed higher limit than the regulatory limit exhibiting 65% followed by 53.5%, 47.5%, 47%, 46.5%, 45%, 28.5%, 27%, 27%, 18% over the standard limit (1 ppm) in the area of Rangpur, Kishoreganj, Gaibandha, Manikganj, Panchagarh, Kustia, Bogura, Nilphamari, Thakurgaon, Lalmonirhat respectively. On the other hand, highest and lowest aflatoxin concentration was recorded in Chuadanga and Dinajpur regions and in terms of percent aflatoxin concentration over standard limit, eight regions were below the permissible limit of aflatoxins, conversely, five regions exposing 915.7% followed by 587.3%, 214.8%, 208.85%, 19.5% aflatoxin concentration beyond permissible limit of 10 µg in the region of Chuadanga, Gaibandha, Kustia, Kishoreganj and Cumilla respectively. Refs. [15, 93] recorded that surges of aflatoxin contamination levels beyond regulatory limit due to increased droughts, pest damages, temperatures, host susceptibility.

As we observed that both aflatoxin and fumonisin concentration were fluctuate one region to another region which have been also monitored that due to association of several significant factors like temperature, water activity, storage conditions, drought, humidity, insect damage, flowering stage, plant characteristics [94–98]. Ref. [48] revealed that aflatoxin production comprised of several factor including existence of certain genes and in intact that means deletions or insertions within the gene regions, crop stress [99] and in fumonisins two factors temperatures and water potential are fundamental to produce fumonisins [99] along with rainfall patterns, longer durations of drought which has been prominent in Mediterranean regions [100–103]. These all conditions significantly impact on the variation of the population of mycotoxin producing fungi both Fusarium and Aspergillus [103]. In our experiment, we recorded over all three regions (Chuadanga, Kishoreganj, Gaibandha) were engendering higher amount of aflatoxins and fumonisins production respectively, thus we speculated in Chuadanga, temperature fluctuation influences the mycotoxin production, in Kishoreganj which exposed with flood and severe water stress and the region Gaibandha with drought problems, these might have the feasible factor for Aspergillus and Fumonisins to produce gigantic amount of mycotoxins compared to other areas. Aflatoxin levels rise as a result of drought, insect damage, and heat during fungal growth [25]. Marasas [104] found that, the presence of fumonisins is linked to weather conditions, with larger instances occurring during hot and dry conditions. Abbas et al. [105] revealed that A. flavus grows supreme around 28–37°C with a humidity level of at least 80%.

Post-harvest factors are also exacerbate mycotoxin production and generate a favorable condition for fungus related to their growth and mycotoxin production and those include storage fungus, insect infestation, contaminant mold respiration, insects and mites, water availability and temperature ultimately deteriorate grain quality [106–108]. As [109] also observed that interaction between these factors triggered the mycotoxigenic species growth, mycotoxin production, niche occupation and competitiveness, [110] also revealed the moisture and surrounding air conditions also influenced mycotoxin production by initiating biological and biochemical activity. Maize is a hygroscopic crop which easily absorbs or release moisture and humidity in the surrounding ambience until getting the adjustment with equilibrium conditions which led to swift degradation in storage. Fusarium species can damage stored grain by causing seedling illnesses, root rots, stalk rots, and ear rots in maize which ultimately hazardous to plants and animal [111–116]. Due to all correlating factors with aflatoxin production, high amount of aflatoxins were found in Bangladeshi markets [23] and 82% contamination in South Asia [49]. Decomposing potentiality of AFs are very slow several approaches including
physical, chemical have been investigated [19] and monitored changing in sensory property and nutrient diminishment which led to mount food safety problems ultimately. A number of microorganisms have been identified fruitfully working as a biocontrol agents to control mycotoxins such as Bacillus subtilis, Pseudomonas, Trichoderma, atoxigenic strains of A. flavus and A. parasiticus [117–119]. Thus, suppressing mycotoxins by biocontrol agent would be a fruitful approach though several experiments need to be conducted precisely in future.

12. Conclusion

Aflatoxins and fumonisins are the major source of disease outbreaks due to a lack of knowledge and consumption of contaminated food and feed in Bangladesh. Excessive levels of aflatoxins and fumonisins in food in Bangladesh is a major concern because still majority of the people have not any idea that they are consuming food and feed which crossed the permissible limit set by EU. Another significant factor is no sign of regulating any acceptable limit for this country and that’s why people are easily contaminated with several mycotoxins without properly knowing any acceptable limit as well as industries are also not ensuring any precise step to diminish mycotoxins concentration in terms of engendering several products. As our study clearly conceded that most of the regions (Rangpur, Gaibandha, Kushtia, Chuadanga, Kishoreganj, Manikganj, Cumilla) were at higher risk for aflatoxin as well as the regions (Panchagarh, Thakurgoan, Nilphamari, Rangpur, Lalmonirhat, Gaibandha, Bogura, Kushtia, Kishoreganj, Manikganj) were exposed with fumonisins contamination more than that of acceptable limit of fumonisins which ultimately effects animal and mankind by entering our food chain. Thus, several effective approaches (physical, chemical, biological, and genetic engineering techniques) need to be employed as early as possible to suppress the ruinous consequences of mycotoxin contamination of Bangladesh.

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Conflict of interest disclosure

Authors do not have any conflict of interests to declare.
References

[1] Aldrich SR, Scott WO, Leng ER. Modern Corn Production. Champaign: A. & L. Publications; 1975

[2] Ranum. Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences. 2014;1312(1):105-112

[3] Ahad MA. Trinojatiyo Fashaler Balai Bebosthapana (Pest Management in Gramicious Crops) (in Bengali). Dhaka, Bangladesh: Textbook Division, Bangla Academy; 2003. p. 184

[4] BBS. Statistical Pocket Book of Bangladesh. Dhaka: Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of the Peoples Republic of Bangladesh; 2019

[5] BBS. Statistical Pocket Book of Bangladesh. Dhaka: Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of the Peoples Republic of Bangladesh; 2020

[6] Covarelli L, Beccari G, Salvi S. Infection by mycotoxigenic fungal species and mycotoxin contamination of maize grain in Umbria, central Italy. Food and Chemical Toxicology. 2011;49:2365-2369

[7] Magan N, Aldred D. Post-harvest control strategies: Minimizing mycotoxins in the field chain. International Journal of Food Microbiology. 2007;119(1-2):131-139

[8] Tsedaley B, Adugna G. Detection of fungi infecting maize (Zea mays L.) seeds in different storages around Jimma, Southwestern Ethiopia. Journal of Plant Pathology and Microbiology. 2016;7:3

[9] Richard JL, Payne GA. Mycotoxins: Risks in Plant, Animal and Human Systems. Ames, IA, USA: Council for Agricultural Science and Technology (CAST); 2003

[10] International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France: International Agency for Research on Cancer; 1993

[11] Food and Agriculture Organization. Worldwide regulations for mycotoxins in food and feed in 2003. In: FAO Food and Nutrition Paper no. 81. Rome: Food and Agriculture Organization; 2004. pp. 1-180

[12] Chu FS. Trichotheccene mycotoxicoses. Encyclopedia. Human Biology. 1997;8:511-522

[13] Reddy SV, Mayi DK, Reddy MU, Thirumala Devi K, Reddy DV. Aflatoxins B1 in different grades of chillies (Capsicum annuum L.) in India as determined by indirect competitive ELISA. Food Additives and Contaminants. 2001;18:553-558

[14] International Agency for Research on Cancer (IARC). Some traditional herbal medicines, somemycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2002;82:1-556

[15] Mehl HL, Jaime R, Callicott KA, Probst C, Garber NP, Ortega-Beltran A, et al. Aspergillus flavus diversity on crops and in the environment can be exploited to reduce aflatoxin exposure and improve health. Belgrade-Zemun, Republic of Serbia: Annals of the New York Academy of Sciences, Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia; 2012:1273-1277

[16] Jaime-Garcia R, Cotty PJ. Crop rotation and soil temperature influence the community structure of Aspergillus flavus in soil. Soil Biology and Biochemistry. 2010;42:1842-1847

[17] Probst C, Cliccott KA, Mehl HL, Jaime R. Aspergillus flavus diversity on crops and in the environment can be
exploited to reduce aflatoxin exposure and improve health. Annals of the New York Academy of Sciences. 2010;1273(1):7-17

[18] Agbetiaimeh D, Ortega-Beltran A, Awuah RT, Atehnkeng J, Cotty PJ, Bandyopadhyay R. Prevalence of aflatoxin contamination in maize and groundnut in Ghana: Population structure, distribution, and toxigenicity of the causal agents. Plant Disease. 2018;102(2018):764-772

[19] Scott PM, Trucksess WM. Mycotoxins in botanicals and dried fruits: A review, Food Additives and Contaminants—Part A Chemistry, Analysis, Control, Exposure and Risk Assessment. 2008;25(2):181-192

[20] Park KY, Bullerman LB. Effect of cycling temperatures on aflatoxin production by Aspergillus parasiticus and Aspergillus flavus in rice and Cheddar cheese. Journal of Food Science. 1983;48:889-896

[21] Abbas HK, Abbas MA, Locke RM, et al. Zablotoicz spatial variability of Aspergillus flavus soil populations under different crops and corn grain colonization and aflatoxins. Canadian Journal of Botany. 2004;82:1768-1775

[22] Lakkireddy K, Lakkireddy K, Kondapalli KS, et al. Aflatoxin in food and feed: The science of safe food. Research & Reviews: Journal of Food Science and Technology. 2014, 2014;3:6-11

[23] Bhuiyan MNH, Hassan MT, Begum M, Ahsan M, Rahim M. Occurrence and seasonal trends of aflatoxin in rice, maize and wheat in Bangladesh. IJSAT. 2013;9(8):1815-1272

[24] Fakruddin M, Chowdhury A, Hossain NM, Ahmed MM. Characterization of aflatoxin producing Aspergillus flavus from food and feed samples. SpringerPlus. 2015;4:159. DOI: 10.1186/s40064-015-0947

[25] Garcia R, Cotty PJ. Aspergillus flavus in soils and corncobs in South Texas: Implications for management of aflatoxins in corn-cotton rotations. Plant Disease. 2004;88:1366-1371

[26] Degola F, Berni E, Dall’Asta C, Spotti E, Marchelli R, Ferrero I, et al. A multiplex RT-PCR approach to detect aflatoxigenic strains of Aspergillus flavus. Journal of Applied Microbiology. 2007;103:409-417. DOI: 10.1111/j.1365-2672.2006.03256.x

[27] Ali N, Hossain K, Blaszkewicz M, Rahman M, Mohanto NC, Alim A, et al. Occurrence of aflatoxin M1 in urine from rural and urban adult cohorts in Bangladesh. Archives of Toxicology. 2016;90:1749-1755

[28] Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS, Blunden G. The occurrence of mycotoxins in key commodities in Bangladesh: Surveillance results from 1993 to 1995. Journal of Natural Toxins. 2002;11:379-386

[29] Roy M, Harris J, Afreen S, Deak E, Gade L, Balajee SA, et al. Aflatoxin contamination in food commodities in Bangladesh. Food Additives & Contaminants. Part B, Surveillance. 2013;6:17-23

[30] Shephard GS, Westhuizen LV, Sewram V. Biomarkers of exposure to fumonisin mycotoxins: A review. Food Additives & Contaminants. 2003;24(10):1196-1201

[31] Glenn KC. Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology: Lysine maize as a case study. Journal of AOAC International. 2007;90:1470-1147

[32] Munkvold GP, Desjardins AE. Fumonisins in maize. Can we reduce their occurrence? Plant Disease. 1997;81:556-564
[33] Sutton JC. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Cymicidirrn Journal of Plant Pathology. 1982; 4:195-209

[34] Rheeder JP, Marasas WF, Vismer H. Production of fumonisin analogs by Fusarium species. Applied and Environmental Microbiology. 2002; 68(5):2101-2105

[35] Jens FC, Jørn S, Robert SA, Thomas LO, Ulf T. Fumonisin B2 production by *Aspergillus niger*. Journal of Agricultural and Food Chemistry. 2007;55(23):9727-9732

[36] Duan C, Qin Z, Yang Z, Li W. Identification of pathogenic Fusarium spp. causing maize ear rot and potential mycotoxin production in China. Toxins. 2016;8(6):186

[37] Alexander NJ, Proctor RH, McCormick SP. Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. Toxin Reviews. 2009;28:198-215. DOI: 10.1080/15569540903092142

[38] Baldwin T, Riley R, Zitomer N, Voss K, Coulombe R Jr, Pestka J, et al. The current state of mycotoxin biomarker development in humans and animals and the potential for application to plant systems. World Mycotoxin Journal. 2011;4(3):257-270

[39] Schmidt-Heydt M, Geisen R. Gene expression as an indication for ochratoxin A biosynthesis in *Penicillium nordicum*. Mycotoxin Research. 2007; 23:13-21

[40] Schmidt-Heydt M, Magan N, Geisen R. Stress induction of mycotoxin biosynthesis genes by abiotic factors. FEMS Microbiology Letters. 2008;284:142-149

[41] De Boevre M, Mavunguose DJ, Landschoot S, Audenaer K. Natural occurrence of mycotoxins and their masked forms in food and feed products. World Mycotoxin Journal. 2012;5(3):207-219

[42] Fanelli F, Iversen A, Logrieco A, Mule G. Relationship between fumonisin production and FUM gene expression in *Fusarium verticillioides* under different environmental conditions, Food Additives and Contaminants—Part A Chemistry, Analysis, Control, Exposure and Risk Assessment. 2012;30(2):365-371

[43] Proctor RH, Van Hove F, Susca A, Stea G, Busman M, Van der Lee T, et al. Birth, death and horizontal gene transfer of the fumonisin biosynthetic gene cluster during the evolutionary diversification of *Fusarium*. Molecular Microbiology. 2013;90:290-306. DOI: 10.1111/mmi.12362

[44] Desjardins AE. *Fusarium* Mycotoxins. Chemistry, Genetics and Biology. St Paul, MN: APS Press; 2006

[45] Battilani P, Camardo LM, Rossi V, Giorni P. AFLA-maize, a mechanistic model for *Aspergillus flavus* infection and aflatoxin B1 contamination in maize. Computers and Electronics in Agriculture. 2013;94:38-46

[46] EFSA. Scientific opinion on the risks for public health related to the presence of zearalenone in food. EFSA Journal. 2011;9(6):2197

[47] Jindal N, Mahipal SK, Rottinghaus GE. Occurrence of fumonisin B1 in maize and poultry feeds in Haryana, India. Mycopathologia. 1999;148:37-40

[48] Jakic-Dimic D, Nestic K. Mycotoxins in feed. In: Proceedings of the XIII Symposium Feed Technology. Novi Sad; 2009;1273(1):7-17

[49] Gruber-Dorninger C, Jenkins T, Schatzmayr G. Global mycotoxin

---

Aflatoxins and Fumonisins Contamination of Maize in Bangladesh: An Emerging Threat...
DOI: http://dx.doi.org/10.5772/intechopen.101647
occurrence in feed: A ten-year survey. Toxins (Basel). 2019;11(7):375

[50] Phillips SI, Wareing PW, Dutta A, Panigrahi S, Medlock V. The mycoflora and incidence of aflatoxin, zearalenone and sterigmatocystin in dairy feed and forage samples from Eastern India and Bangladesh. Mycopathologia. 1996; 133(1):15-21

[51] Giasuddin M, Sil BK, Alam J, Koike I, Islam MR, Rahman MM. Prevalence of poultry diseases in Bangladesh. Journal of Biological Sciences. 2002;2:212-213

[52] Abdel-Wahhab MA, Abdel-Galil MM, Hassan AM, Hassan NH, Nada SA, Saeed A, et al. Zizyphus spin-x-christi extract protects against aflatoxin B1-intitiated hepatic carcinogenicity. African Journal of Traditional, Complementary, and Alternative Medicines. 2007;4:248-256

[53] Tejada AW, Rustia A. Risk assessment of contaminants in foods: Mycotoxins and pesticide residues. In: Proceedings of FFTCeKU 2011 Conference, International Seminar on Risk Assessment and Risk Management of Mycotoxins for Food Safety in Asia. Thailand: Kasetsart University; 2011

[54] Yoshizawa T. A current situation of mycotoxin management in Asia in relation to recent actions in Japan. In: Proceedings of FFTCeKU 2011 Conference, International Seminar on Risk Assessment and Risk Management of Mycotoxins for Food Safety in Asia. Thailand: Kasetsart University; 2011

[55] Mahfuz M, Alam MA, Fahim SM, Jyoti MR, Hossain M, Egner PA, et al. Aflatoxin exposure in children living in Mirpur, Dhaka: Data from MAL-ED companion study. Journal of Exposure Science & Environmental Epidemiology. 2019;29:655-662

[56] Van Egmond HP, Schoothorst RC, Jonker MA. Regulations relating to mycotoxins in food. Analytical and Bioanalytical Chemistry. 2007;389: 147-157

[57] Moss MO. Mycotoxin review—2 Fusarium. Mycologist. 2002;16:158-161

[58] Ahlberg SH, Joutsjoki V, Korhonen HJ. Potential of lactic acid bacteria in aflatoxin risk mitigation. International Journal of Food Microbiology. 2015;207:87-102

[59] Stevens DB, Turner JA, Paveley ND. Exploiting variety resistance to rationalise fungicide inputs—Theory and practice. Aspects of Applied Biology. 1997;50:279-284

[60] Missmer SA, Suarez L, Felkner M, Wang E, Merrill AH Jr, Rothman KJ, et al. Exposure to fumonisins and the occurrence of neural tube defects along the Texas-Mexico border. Environmental Health Perspectives. 2006;114:237-241

[61] Covarelli L, Stifano S, Beccari G, Raggi L, Lattanzio VMT, Albertini E. Characterization of Fusarium verticillioides strains isolated from maize in Italy: Fumonisin production, pathogenicity and genetic variability. Food Microbiology. 2012;31:17-24

[62] Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggaar R, et al. Fumonisins-Novel mycotoxins with cancer-promoting activity produced by Fusarium moniliforme. Applied and Environmental Microbiology. 1988;54:1806-1811

[63] Turna NS, Wu F. Risk assessment of aflatoxin-related liver cancer in Bangladesh. Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment. 2019;36(2):320-326

[64] ISTA. International Rules for Seed Testing. Bassersdorf, Switzerland: International Seed Testing Association; 2006
[65] Sreenu B, Girish A, Alice J, Sujeetha R. Identification and detection of maize seed borne pathogens using different seed testing methods. International Journal of Current Microbiology and Applied Sciences. 2019;8:1460-1466

[66] Dinu D, Nechifor MT, Stoian G, Costache M, Dinischiotu A. Enzymes with new biochemical properties in the pectinolytic complex produced by Aspergillus niger MIUG 16. Journal of Biotechnology. 2007;131:128-137

[67] Mlakar T, Legiša M. Citrate inhibition-resistant form of 6-phosphofructo-1-inase from Aspergillus niger. Applied and Environmental Microbiology. 2006;72:4515-4521

[68] Probst AV, Dunleavy E, Almouzni G. Epigenetic inheritance during the cell cycle. Nature Reviews. Molecular Cell Biology. 2009;10:192-206

[69] Navi SS, Bandypadhyay R, Hall AJ, Bramel-Cox PJ. A pictorial guide for the identification of mold fungi on sorghum grain. In: Information Bulletin no. 59. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; 1999. 118 pp

[70] White TJ, Bruns TD, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal genes form phylogenetics. In: Innis MA, Gelfrand DH, Sninsky JJ, White TJ, editors. PCR Protocols. San Diego, California, USA: Academic Press; 1990. pp. 315-322

[71] Criseo G, Bagnara A, Bisignano G. Differentiation of aflatoxin-producing and non-producing strains of Aspergillus flavus group. Letters in Applied Microbiology. 2001;33:291-295

[72] Bluhm BM, Cousin MA, Woloshuk CP. Multiplex real-time PCR detection of fumonisin-producing and trichothecene-producing groups of Fusarium species. Journal of Food Protection. 2004;67:536-543

[73] Shapira R, Paster N, Eyal O, Menasherov M, Mett A, Salomon R. Detection of aflatoxigenic molds in grains by PCR. Applied and Environmental Microbiology. 1996;62:3270-3273

[74] Chen RS, Tsay JG, Huang YF, Chiu RYY. Polymerase chain reaction mediated characterization of molds belonging to the Aspergillus flavus group and detection of A. parasiticus in peanut kernels by multiplex polymerase chain reaction. Journal of Food Protection. 2002;65:840-844

[75] Mayer Z, Bagnara A, Färber P, Geisen R. Quantification of the copy number of nor-1, a gene of the aflatoxin biosynthetic pathway by real-time PCR, and its correlation to the cfu of Aspergillus flavus in foods. International Journal of Food Microbiology. 2003;82:143

[76] Ibrahim F, Jalal H, Khan AB, Asghar MA, Iqbal J, Ahmed A, et al. Prevalence of aflatoxigenic Aspergillus in food and feed samples from Karachi, Pakistan. JIMB. 2016;4(1):1-8

[77] Levin ER. PCR detection of aflatoxin producing fungi and its limitations. International Journal of Food Microbiology. 2012;156:1-6

[78] Ana A, Savoie J-M, Chereau S, Ducos C, Aguilar M, et al. Primingto protect maize from Fusarium verticillioides and its fumonisin accumulation. Journal of the Science of Food and Agriculture. 2019;99(1):64-72

[79] Monika R, Julie H, Sadia A, Eszter D, Lalitha G, S. A. B., & Stephen, L. Aflatoxin contamination in food commodities in Bangladesh. Food Additives & Contaminants: Part B: Surveillance. 2013;6(1):17-23. DOI: 10.1080/19393210.2012.720617
Maize - Recent Advances, Applications and New Perspectives for Crop Improvement

[80] Rheeder JP, Marasas WFO, van Schalkwyk DJ. Incidence of *Fusarium* and *Diploidia* species in naturally infected grain of South African maize cultivars: A follow-up study. Phytophylactica. 1993;25:43-48

[81] Walayar F, Reddy SV, Lava-Kumar P. Review of immunological methods for the quantification of aflatoxins in peanut and other foods. Peanut Science. 2009;36:54-59. DOI: 10.3146/AT07-007.1

[82] Ketney O, Santini A, Oancea E. Recent Aflatoxin survey data in milk and milk products: A review. International Journal of Dairy Technology. 2017;70:320-331. DOI: 10.1111/1471-0307.12382

[83] Andreasson U, Perret-Liaudet A, Waalvijk van Doorn LJC, Perret-Liaduet A, Blennov K, Chiasserini D, et al. A practical guide to immunoassay method validation. Frontiers in Neurology. 2015;6:179

[84] Czéh Á, Mandy F, Feher-Toth S, Torok L, Mike Z, Koszegi B, et al. A flow cytometry based competitive fluorescent microsphere immunoassay (CFIA) system for detecting up to six mycotoxins. Journal of Immunological Methods. 2012;384:71-80. DOI: 10.1016/j.jim.2012.07.010

[85] Czéh Á. Mikrogyöngy Alapú Multiplex Immunoassay Rendszer Fejlesztése Multi-mikotoxin Vizsgálatra: Túl a XX. Századi Alkalmazásokon [master’s thesis, Interdiszciplináris Orvostudományok Doktori Iskola D93]. Pécs: University of Pécs; 2014

[86] Bánáti H, Darvas B, Fehér-Tóth S, Czéh Á, Székács A. Determination of mycotoxin production of *Fusarium* species in genetically modified maize varieties by quantitative flow immunocytometry. Toxins. 2017;9:70. DOI: 10.3390/toxins9020070

[87] Kumar Ajith K, Naik MK. Prevalence and distribution of aflatoxin contamination of chilli (*Capsicum annuum* L.) field and market. Karnataka. The Journal of Agricultural Science. 2005;18(2):520-523

[88] Lopez-Erasquin E, Vazquez C, Jimenez M, Gonzalez-Jaen MT. Real-time RT-PCR assay to quantify the expression of *fum1* and *fum19* genes from the fumonisin-producing *Fusarium verticillioides*. Journal of Microbiological Methods. 2007;68:312-317

[89] Williams WP. Breeding for resistance to aflatoxin accumulation in maize. Mycotoxin Research. 2006;22:27-32

[90] Saito M, Machida S. A rapid identification method for aflatoxin producing strains of *A. flavus* and *A. parasiticus* by ammonia vapor. Mycoscience. 1999;40:205-222

[91] Bennett JW, Lee LS. Mycotoxins—Their biosynthesis in fungi: Aflatoxins and other bisfuranooids. Journal of Food Protection. 1979;42:805-809

[92] Gary M. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. European Journal of Plant Pathology. 2003, 2003;109(7):705-713

[93] Maiorano A, Reyneri A, Magni A, Ramponi C. A decision tool for evaluating the agronomic risk of exposure to fumonisins of different maize crop management systems in Italy. Agricultural Systems. 2009;102:17-23

[94] Cao A, Santiago R, Ramos AJ, Souto XC, Aguin O, Malvar RA, et al. Critical environmental and genotypic factors for *Fusarium verticillioides* infection, fungal growth and fumonisin contamination in maize grown in northwestern Spain. International Journal of Food Microbiology. 2014;177:63-71. DOI: 10.1016/j.ijfoodmicro.2014.02.004

[95] Czembor E, Stepien Ł, Waśkiewicz A. Effect of environmental
factors on *Fusarium* species and associated mycotoxins in maize grain grown in Poland. PLoS One. 2015;10:e0133644. DOI: 10.1371/journal.pone.0133644

[96] Cendoya E, Chiotta ML, Zachetti V, Chulze SN, Ramirez ML. Fumonisins and fumonisin-producing *Fusarium* occurrence in wheat and wheat by products: A review. Journal of Cereal Science. 2018;80:158-166

[97] Chen ZY, Brown RL, Cleveland TE. Evidence for an association in corn between stress tolerance and resistance to *Aspergillus flavus* infection and aflatoxin contamination. African Journal of Biotechnology. 2004;3:693-699

[98] Magan N, Aldred D. Why do fungi produce mycotoxins? In: Dijkstra J, Samson R, editors. Food Mycology: A Multifaceted Approach to Fungi and Food. Boca Raton, FL, USA: CRC Press; 2007

[99] Jurado M, Vázquez C, Callejas C, González-Jaén M. Occurrence and variability of mycotoxigenic *Fusarium* species associated to wheat and maize in the South West of Spain. Mycotoxin Research. 2006;22:87-91

[100] Aliakbari F, Mirabolfathy M, Emami M, Mazhar SF, Karami-Osboo R. Natural occurrence of *Fusarium* species in maize kernels at Gholesan province in northern Iran. Asian Journal of Plant Sciences. 2007;8:1276-1281

[101] Cavagliere L, Keller K, Pereyra C, Pereyra MG, Alonso V, Rojo F, et al. Fungi and natural incidence of selected mycotoxins in barley rootlets. Journal of Stored Products Research. 2009;45:147-150

[102] Gil-Serna J, Mateo E, González-Jaén M, Jiménez M, Vázquez C, Patiño B. Contamination of barley seeds with *Fusarium* species and their toxins in Spain: An integrated approach. Food Additives & Contaminants: Part A. 2013;30:372-380

[103] Magan N, Medina A, Aldred D. Possible climate-change effects on mycotoxin contamination of food crops pre and postharvest. Plant Pathology. 2011;60:150-163

[104] Marasas FO, W. Discovery and occurrence of the fumonisins: A historical perspective. Environmental Health Perspectives. 2001;109(suppl. 2):239-243

[105] Abbas HK, Zablotowicz RM, Bruns HA. Modeling of colonization of maize by toxigenic and non-toxigenic *Aspergillus flavus* strains: Implication for biological control. World Mycotoxin Journal. 2008;1:333-340

[106] Jian F, Jayas DS. The ecosystem approach to grain storage. Agricultural Research. 2012;1(2):148-156

[107] Johnson LA. Corn production, processing and utilization. In: Lorenz KJ, Kulp K, editors. Handbook of Cereal Science and Technology. Vol. 1. New York, U.S.A: Marcel Dekker Inc; 1991. pp. 55-131

[108] Magan N, Hope R, Cairns V, Aldred D. Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. European Journal of Plant Pathology. 2003;109(7):723-730

[109] Jayas DS, White Noel DG. Storage and drying of grain in Canada: Low cost approaches. Food Control. 2003;14(4):255-261

[110] Placinta C, D’Mello JP, Macdonald AM. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Animal Feed Science and Technology. 1999;78:21-37

[111] Bennett JW, Klich M. Mycotoxins. Clinical Microbiology Reviews. 2003;16:497-516
[112] Richard JL. Some major mycotoxins and their mycotoxicoses—An overview. International Journal of Food Microbiology. 2007;119:3-10

[113] Streit E, Schatzmayr G, Tassis P, Tzika E, Marin D, Taranu I, et al. Current situation of mycotoxin contamination and co-occurrence in animal feed—Focus on Europe. Toxins. 2012;4:788-809

[114] Bryden WL. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Animal Feed Science and Technology. 2012;173:134-158

[115] Arunachalam C, Doohan FM. Trichothecene toxicity in eukaryotes: Cellular and molecular mechanisms in plants and animals. Toxicology Letters. 2013;217:149-158

[116] Abbas HK, Yoshizawa T, Shier WT. Cytotoxicity and phytotoxicity of trichothecene mycotoxins produced by Fusarium spp. Toxicon. 2013;74:68-75

[117] Bhattacharjee R, Dey U. An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. African Journal of Microbiology Research. 2014;8:1749-1762

[118] Mukhopadhyay R, Kumar D. Trichoderma: A beneficial antifungal agent and insights into its mechanism of biocontrol potential. Egyptian Journal of Biological Pest Control. 2020;30:133

[119] Dorner JW, Cole RJ. Effect of application of nontoxigenic strains of Aspergillus flavus and A. parasiticus on subsequent aflatoxin contamination of peanuts in storage. Journal of Stored Products Research. 2002;38:329-339