

ASSESSMENT OF AFLATOXIN B1 CONTAMINATION AND RISK FACTORS IN MAIZE-BASED POULTRY FEED IN BANGLADESH

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ABSTRACT

Maize is a major component in Bangladeshi poultry feeds yet is susceptible to aflatoxigenic fungal infection. This paper estimated the prevalence of Aflatoxin B1 (AFB1) in maize in Dinajpur and identified post-harvest risk factors for contamination. A cross-sectional survey was conducted on 120 maize samples from 13 upazilas of Dinajpur. The Competitive Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the AFB1 levels. Feed moisture content, storage time, insect infestation, and ventilation were recorded. Fungal isolates were cultured and molecularly confirmed for toxigenic potential via PCR amplification of four aflatoxin biosynthesis genes (*aflR*, *omt-A*, *ver-1*, and *nor-1*). Overall, 23.3% of samples exceeded the 5.0 µg/kg international safety standard (maximum: 124.3 µg/kg), with strong seasonal variation (40.0% in summer vs. 6.7% in winter). Multivariable analysis showed that moisture content >15.0% (adjusted OR = 21.34) and insect infestation (adjusted OR = 5.72) independently predicted AFB1 contamination. Of 73 positive fungal isolates, 71.2% were *aflR* regulatory gene-positive, and 6.4% had all four biosynthetic genes, suggesting high toxigenic potential. Maize AFB1 contamination indicates gene-environment interactions. High-risk *Aspergillus* strains are abundant in the supply chain and particularly active during warm, wet seasons. Keeping grain moisture below 15.0% and adopting integrated pest management can greatly reduce contamination risk.

Keywords: Aflatoxin B1, Poultry feed, *Aspergillus flavus*, Moisture content, Maize, Bangladesh.

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1. INTRODUCTION

Maize (*Zea mays* L.) is a major cereal ingredient in poultry feed formulations, often accounting for 50–60% of the total diet and serving as the principal source of dietary energy (Nizamlyoğlu & Oguz, 2003; Abbas et al., 2024; Syamsu et al., 2025a; Haris et al., 2026). The continued expansion of poultry production has increased the demand for high-quality feed ingredients to support animal health, productivity, and feed efficiency (Yalew et al., 2026). However, maize's nutritional composition, particularly its high starch content, and its susceptibility to moisture absorption during storage make it vulnerable to fungal colonization and mycotoxin contamination. Such contamination can compromise feed quality and adversely affect poultry health and production performance (Akbar et al., 2020; Joshi et al., 2022; Syamsu et al., 2025b).

Among maize-associated mycotoxins, aflatoxins are of particular concern because of their toxicity, widespread occurrence, and implications for animal and human health. These secondary metabolites are produced primarily by aflatoxigenic *Aspergillus* species, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, under environmental conditions that favor fungal growth and toxin biosynthesis (Akbar et al., 2020; Haque et al., 2020; Yalew et al., 2026). Aflatoxins comprise a group of structurally related compounds, of which aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) are among the most extensively studied. These compounds are named for their characteristic blue or green fluorescence under ultraviolet light (Kepka-Borkowska et al., 2025). Aflatoxigenic strains of *A. flavus* generally produce B-type aflatoxins, whereas *A. parasiticus* can produce both B- and G-type aflatoxins (Zeweld et al., 2025; Syamsu et al., 2026a). Among these compounds, AFB1 is particularly important because of its potent

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hepatotoxic, mutagenic, and carcinogenic properties. The International Agency for Research on Cancer has classified it as a Group 1 human carcinogen (Kępka-Borkowska et al., 2025; Zeweld et al., 2025). Consequently, regulatory authorities in different countries have established maximum permissible levels for aflatoxins in food and feed, although the applicable limits vary according to the commodity, intended use, and regulatory jurisdiction.

Aflatoxin B1 contamination in poultry feed poses substantial risks to animal health and production efficiency. The liver is a principal target organ of AFB1 toxicity, and exposure may cause hepatic enlargement, cellular injury, and, under prolonged or severe conditions, pathological changes such as fibrosis (Monson et al., 2014). Chronic exposure can also impair immune function by adversely affecting lymphoid organs, including the bursa of Fabricius, thymus, and spleen, potentially compromising both humoral and cell-mediated immune responses and increasing susceptibility to infectious diseases (Peng et al., 2015; Fouad et al., 2019). Other reported consequences include reduced feed intake, impaired growth, poorer feed conversion efficiency, and increased mortality, depending on the exposure level and duration (Chupia et al., 2022; Yalew et al., 2026). In laying hens, prolonged exposure may additionally reduce egg production and adversely affect egg quality (Wei et al., 2024). These effects can cause considerable economic losses through reduced productivity, increased disease susceptibility, and added costs for feed quality management and animal health.

Beyond its effects on poultry, AFB1 contamination raises concerns about food safety and human health. Dietary exposure to aflatoxins is strongly associated with adverse health outcomes, particularly hepatocellular carcinoma, and may contribute to other health problems in populations experiencing prolonged exposure (Mitchell et al., 2017; Saha Turna & Wu, 2019; Hassen et al., 2024). In poultry, AFB1 and certain metabolites may transfer from contaminated feed into edible tissues and eggs, although the extent of carryover varies by species, exposure conditions, metabolism, and food matrix (Tarannum et al., 2020). Thus, contaminated feed may represent an indirect route of human exposure, in addition to the more direct risks associated with consumption of contaminated cereals and other food commodities. Surveys across regions have reported considerable variation in aflatoxin occurrence and concentrations in maize and maize-based products, reflecting differences in agricultural practices, climatic conditions, post-harvest handling, and storage management (Yohannis et al., 2025). These observations underscore the importance of controlling aflatoxin contamination throughout the maize supply chain.

Aflatoxin contamination results from complex interactions among fungal characteristics, environmental conditions, and post-harvest management practices. Temperature, relative humidity, grain moisture content, and storage duration strongly influence fungal growth and aflatoxin production. Warm, humid conditions, especially with inadequate grain drying and prolonged storage, can favor aflatoxigenic fungi and increase the likelihood of toxin accumulation (Mannaa & Kim, 2018; Mongkon et al., 2017; Joshi et al., 2022; Chupia et al., 2022). Grain moisture content exceeding approximately 15% has been identified as a potential risk factor under certain storage conditions, although the threshold for aflatoxin production depends on temperature, water activity, fungal strain, and other environmental variables. Insect infestation, poor sanitation, inadequate ventilation, and limited access to appropriate storage infrastructure may further increase contamination risks, particularly in smallholder production and marketing systems (Mannaa & Kim, 2018; Yalew et al., 2026).

Evidence from several developing regions indicates that aflatoxin contamination remains a persistent challenge in maize production and storage. Studies in Uganda and Pakistan reported substantial contamination of maize and feed ingredients, including samples that exceeded applicable safety limits (Akbar et al., 2020; Nakavuma et al., 2020). Similarly, a recent review of aflatoxin contamination in sub-Saharan Africa emphasized maize as a potential dietary exposure source and highlighted its public health implications (Bereda, 2026). These findings illustrate how poor storage conditions and inadequate post-harvest management can increase aflatoxin exposure. However, contamination prevalence and concentrations vary considerably across regions and studies, making location-specific surveillance essential for identifying relevant risk factors and designing effective mitigation strategies.

Beyond environmental conditions, the genetic characteristics of *Aspergillus* populations influence their ability to produce aflatoxins. Aflatoxin biosynthesis involves a coordinated pathway regulated by multiple structural and regulatory genes. The presence, absence, or variation of these genes may contribute to differences in the aflatoxigenic potential of individual isolates. Recent molecular investigations of *A. flavus* recovered from field maize in Bangladesh have identified genes associated with aflatoxin biosynthesis and reported variation in their detection among isolates (Sultana et al., 2026). Such findings demonstrate the value of molecular approaches for investigating the genetic potential of fungal isolates. Nevertheless, detecting aflatoxin biosynthetic genes does not necessarily indicate active gene expression or toxin production, because environmental conditions and regulatory mechanisms also influence aflatoxin biosynthesis. Accordingly, interpret molecular characterization alongside direct measurements of aflatoxin contamination to better understand potential risks.

Bangladesh presents environmental and agricultural conditions that may favor aflatoxin contamination during maize production, handling, and storage. Its tropical monsoon climate features high temperatures, seasonal rainfall, and elevated humidity, particularly during the monsoon, which can support fungal growth when grain drying and

storage are inadequate (Tarannum et al., 2020). The country's poultry feed supply chain includes numerous small-scale producers, traders, and feed-processing operations, some of which may have limited access to modern storage facilities, moisture-monitoring equipment, and standardized post-harvest management practices (Yamin et al., 2025). These conditions may increase maize vulnerability to fungal contamination during storage and distribution.

Previous investigations in Bangladesh have documented AFB1 contamination in maize collected from different geographical locations, with reported contamination prevalence ranging from 3.2% to 100% across studies and mean concentrations of approximately 23.47–32.34 µg/kg in the cited investigations (Lubna et al., 2018; Kamal et al., 2022). The wide variation may reflect differences in sampling locations, seasons, storage conditions, analytical methods, and contamination thresholds. Although these studies provide valuable evidence of aflatoxin contamination in Bangladesh, information remains limited on how post-harvest storage conditions, seasonal variation, and the molecular characteristics of aflatoxigenic *Aspergillus* isolates interact in Dinajpur District, northwestern Bangladesh.

Despite growing awareness of aflatoxin-related risks, important gaps remain in understanding how storage environments, fungal populations, and AFB1 contamination relate under practical maize-handling conditions. Previous studies have examined environmental risk factors, such as moisture content, temperature, and storage duration, or investigated the genetic characteristics of aflatoxigenic fungi. However, fewer investigations have integrated field-based assessments of post-harvest conditions with molecular characterization of aflatoxin biosynthetic genes and direct quantification of AFB1 in maize samples (Akullo et al., 2023; Sana et al., 2025). This integrated approach is particularly relevant to smallholder storage systems, where environmental conditions and management practices can vary widely. Furthermore, although seasonal variation in aflatoxin contamination has been documented, additional location-specific quantitative evidence is needed to determine how contamination patterns differ across sampling periods and storage conditions.

Therefore, the present study aimed to determine the occurrence and concentrations of AFB1 in maize associated with poultry feed supply chains in Dinajpur District, Bangladesh, and to examine their relationships with post-harvest factors, including storage duration, grain moisture content, ventilation, and pest infestation. The study also examined variation in AFB1 contamination across sampled seasons and characterized selected aflatoxin biosynthetic genes in *Aspergillus* isolates recovered from maize. By integrating field-based contamination data, storage-related risk factors, and molecular characterization, this study aimed to identify potential contamination risks and inform practical, locally appropriate aflatoxin mitigation strategies in maize-based poultry feed systems.

2. MATERIALS AND METHODS

2.1. Study Area and Design

A cross-sectional study was conducted in Dinajpur District, northwestern Bangladesh (25.6261° N, 88.6346° E). The district experiences marked seasonal variation in climatic conditions, with temperatures ranging from approximately 15°C during winter (November–February) to 32°C during summer (May–August). Relative humidity varies seasonally from approximately 35% to 85% (Climate-Data.org). Dinajpur comprises 13 upazilas (subdistricts) with diverse agroecological conditions and grain-storage practices. Maize is widely cultivated in the district, partly because of its economic advantages over alternative crops.

Supplementary Table 1: Distributions of Samples

S/N	Upazila	Number of Samples		
		Winter	Summer	Total
1	Dinajpur Sadar	5	5	10
2	Chirir Bandar	4	5	9
3	Kaharole	5	4	9
4	Biral	5	5	10
5	Bochaganj	4	5	9
6	Khansama	5	4	9
7	Birganj	5	5	10
8	Parbatipur	5	4	9
9	Fulbari	4	5	9
10	Birampur	5	4	9
11	Nawabganj	4	5	9
12	Ghoraghat	5	4	9
13	Hili	4	5	9
Total		60	60	120

2.2. Sampling Strategy and Sample Collection

A stratified cross-sectional sampling design was employed to investigate seasonal differences in aflatoxin B1 (AFB1) contamination of maize. A total of 120 maize samples were collected from storage and marketing facilities across the 13 upazilas of Dinajpur District (Fig. 1; Supplementary Table 1). Sampling locations included farmer-operated storage facilities, small-scale feed mills, cooperative associations, and grain traders, representing important components of the local maize supply chain.

Samples were collected during two distinct seasonal periods: winter (January–February; n = 60) and summer (June–July; n = 60). Sampling was distributed across the 13 upazilas during each period to

provide geographical coverage and facilitate comparisons of AFB1 contamination between seasons.

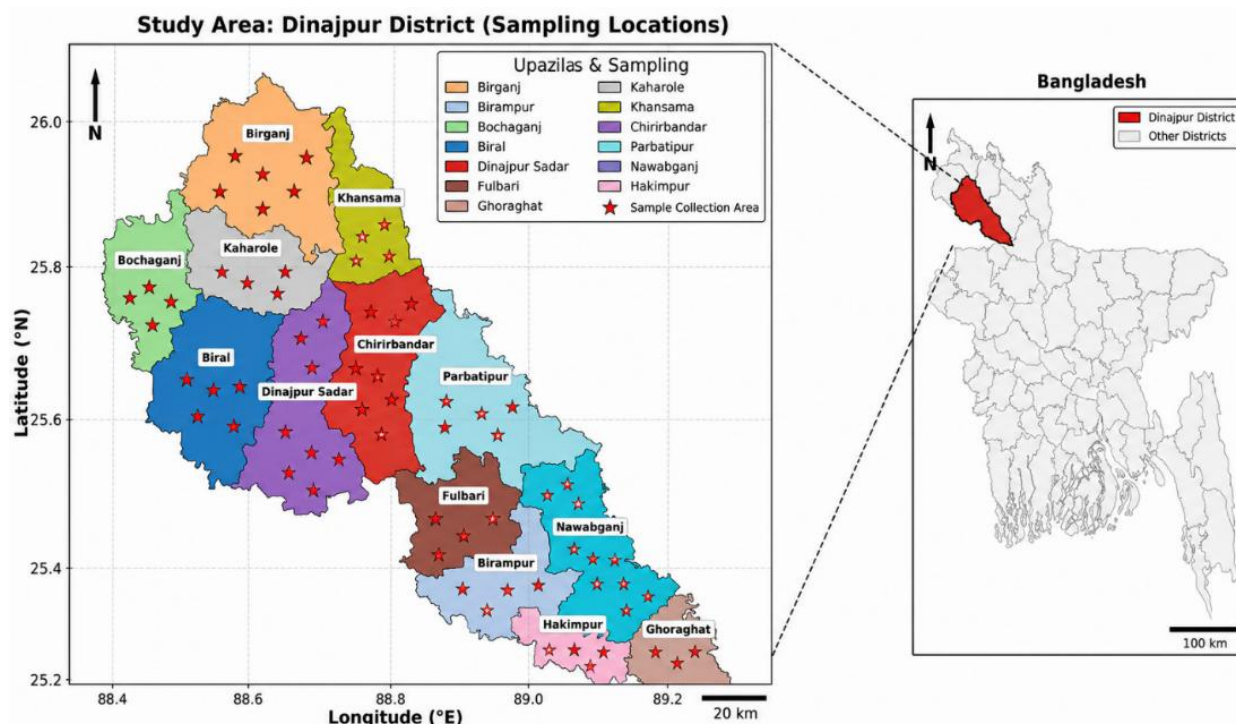


Fig. 1: Study Map of Sampling Location in Dinajpur district.

At each sampling location, we collected maize grain from selected storage bags, grain lots, or storage bins and combined it to obtain an approximately 100-g composite sample. Samples were aseptically transferred into sterile containers and transported to the Microbiology Laboratory at Hajee Mohammad Danesh Science and Technology University within 4–6 h of collection. Samples were processed immediately upon arrival or stored at 4°C until analysis.

2.3. Assessment of Storage Conditions and Potential Risk Factors

Information on post-harvest handling and storage practices was collected through structured questionnaires administered to facility operators at the time of sample collection. The assessment included grain moisture content, storage facility type, storage duration, insect infestation, and ventilation conditions.

Storage facilities were categorized as improved facilities with ventilation or traditional open-storage systems. Storage duration was recorded in days from harvest to sampling. Visible insect infestation was classified as present or absent, while ventilation conditions were categorized as adequate, partial, or poor. Grain moisture content was determined separately using the gravimetric method described below.

2.4. Determination of Grain Moisture Content

Moisture content was determined using a gravimetric oven-drying method at 105°C, following the analytical procedure described in the study protocol.

Approximately 5 g of ground, homogenized maize was weighed into an aluminum dish and dried in a hot-air convection oven at 105°C for 18 h. After drying, the sample was cooled in a desiccator for 30 minutes and reweighed. Moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where W_1 represents the initial sample mass before drying and W_2 represents the sample mass after drying.

Based on the measured moisture content, samples were classified into three categories: dry ($\leq 12\%$), intermediate ($>12\text{--}15\%$), and high moisture ($>15\%$). These categories were used to examine the relationship between grain moisture and AFB1 contamination.

2.5. Fungal Isolation and Morphological Identification

Fungi were isolated from maize samples using serial dilution and culture techniques. Briefly, 10 g of ground maize was suspended in 90 mL of sterile 0.85% sodium chloride solution, thoroughly mixed, and serially diluted

to 10^{-5} . Aliquots of 100 μL from appropriate dilutions were spread onto Sabouraud Dextrose Agar (SDA; HiMedia, India) to determine the culturable fungal population. Plates were incubated at 30°C for 5 days in the dark. Following incubation, fungal colonies were counted, and the results were expressed as colony-forming units per gram (CFU/g) of maize.

For the presumptive isolation of *A. flavus* and *A. parasiticus*, samples were cultured on Aspergillus Flavus and Parasiticus Agar (AFPA; HiMedia, India). Plates were incubated at 30°C for 5 days. Colonies exhibiting characteristic orange-to-yellow reverse pigmentation and morphological features consistent with the target *Aspergillus* species were selected for further examination.

Presumptive identification was based on colony characteristics and microscopic morphology. Selected colonies were purified by single-colony isolation and transferred to fresh SDA slants for maintenance and subsequent genomic DNA extraction (Frisvad & Larsen, 2016).

2.6. Molecular Characterization of Aflatoxin Biosynthetic Genes

2.6.1. Genomic DNA Extraction: Genomic DNA was extracted from selected fungal isolates using the TIANamp Genomic DNA Kit (DP304-02; TIANGEN Biotech, China), following the manufacturer's protocol with modifications.

Approximately 50–100 mg of fungal mycelium was aseptically collected from actively growing, 5-day-old SDA cultures and transferred into a 1.5-mL microcentrifuge tube. Subsequently, 200 μL of GB lysis buffer and 20 μL of proteinase K were added. The mixture was thoroughly mixed, frozen in liquid nitrogen for 5 minutes, and mechanically disrupted using a sterile mortar and pestle to facilitate fungal cell lysis.

The resulting suspension was incubated at 70°C for 10 minutes and centrifuged at 12,000 rpm for 30 s. After lysis, 200 μL of 96–100% ethanol was added, and the mixture was vortexed for 15 s.

The lysate was transferred to a CB3 spin column placed in a 2-mL collection tube and centrifuged at 12,000 rpm for 30 s. The flow-through was discarded, and the column was washed sequentially with 500 μL of GD buffer and 600 μL of PW buffer, with centrifugation at 12,000 rpm for 30 s after each wash. A second wash with PW buffer was performed, followed by centrifugation at 12,000 rpm for 2 minutes to remove residual wash solution.

The column was then transferred to a clean 1.5-mL microcentrifuge tube, and 100 μL of TE elution buffer was added directly to the membrane. After incubation at room temperature for 5 minutes, DNA was eluted by centrifugation at 12,000 rpm for 2 minutes. The extracted genomic DNA was stored at -20°C until polymerase chain reaction (PCR) analysis.

2.6.2. PCR Amplification of Aflatoxin Biosynthetic Genes: Four genes associated with the aflatoxin biosynthetic pathway were targeted for PCR amplification: *aflR*, a regulatory gene (expected amplicon size: 1,032 bp); *omtA*, encoding an O-methyltransferase (797 bp); *ver-1*, associated with an intermediate step in aflatoxin biosynthesis (537 bp); and *nor-1*, encoding a norsolorinic acid reductase (400 bp) (Caceres et al., 2020).

These genes were selected to assess the presence of regulatory and structural components associated with aflatoxin biosynthesis. Their detection was interpreted as evidence of the genetic potential for aflatoxin production rather than direct confirmation of toxin synthesis.

PCR amplification was performed in a total reaction volume of 25 μL containing approximately 50 ng of genomic DNA, 0.5 μM each of forward and reverse primers (TIANGEN Biotech, China; Table 1), 12.5 μL of $2\times$ Taq PCR Master Mix, and nuclease-free water to the final reaction volume (Supplementary Table 2). The reported master mix contained Taq DNA polymerase, Tris–HCl buffer, KCl, MgCl_2 , and deoxynucleotide triphosphates (dNTPs). Amplification was conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, USA). The cycling conditions consisted of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 67°C for 30 s, and extension at 72°C for 30 s. A final extension was performed at 72°C for 10 minutes (Supplementary Table 3). An *A. flavus* reference strain (ATCC 16883) was included as the reported positive control, while nuclease-free water served as the negative control.

Table 1: Primers used in this study

Target gene	Primer	Sequence (5'–3')	Amplicon size (bp)	Reference
<i>nor-1</i>	NorF	ACCGCTACGCCGCACTCTCGGCAC	400	Ali et al., 2022
	NorR	GTTGGCCGCCAGCTTCGACACTCCG		
<i>ver-1</i>	VerF	GCCGCAGGCCGCGGAGAAAGTGGT	537	
	VerR	GGGGATATACTCCC GCGACACAGCC		
<i>omt-A</i>	OmtF	GTGGACGGACCTAGTCCGACATCAC	797	
	OmtR	GTCGGCGCCACGCACTGGGTTGGGG		
<i>aflR</i>	AflrF	TATCTCCCCCGGGCATCTCCCGG	1,032	
	AflrR	CCGTCAGACAGCCACTGGACACGG		

Supplementary Table 2: PCR reaction mixture used for amplification of aflatoxin biosynthetic pathway-associated genes

S/N	Component	Volume per reaction (μL)
1	Taq 2× Master Mix with Dye v2	12.5
2	Forward primer	1.0
3	Reverse primer	1.0
4	Template DNA	4.0
5	RNase-free water	6.5
	Total volume	25.0

Supplementary Table 3: PCR thermal cycling conditions used for target gene amplification

PCR step	Temperature	Time	Number of cycles
Initial denaturation	94°C	5 minutes	1
Denaturation	94°C	30 s	35
Annealing	67°C	30 s	35
Extension	72°C	30 s	35
Final extension	72°C	10 minutes	1

2.6.3. Agarose Gel Electrophoresis and Amplicon Detection: PCR products were separated by horizontal electrophoresis using 1.5–1.75% agarose gels prepared in 1× Tris–acetate–EDTA (TAE) buffer containing SYBR Green at the reported dilution of 1:10,000.

A 100-bp DNA ladder (Fermentas, Thermo Fisher Scientific, USA) was used as the molecular size marker. Approximately 5 μL of each PCR product was loaded into the designated wells. Electrophoresis was performed at 45 V for 45 minutes using a horizontal electrophoresis system.

Following electrophoresis, DNA bands were visualized under ultraviolet illumination using a Gel Doc EZ documentation system (Bio-Rad, USA), and digital images were captured for analysis.

Amplification products were identified by comparing their migration positions with the molecular size marker. The expected fragment sizes were 400 bp for *nor-1*, 537 bp for *ver-1*, 797 bp for *omtA*, and 1,032 bp for *aflR*.

Isolates were subsequently grouped according to the number and combinations of aflatoxin biosynthetic genes detected. Gene profiles were recorded to examine variation in the presence of regulatory and structural genes among the fungal isolates.

2.7. Quantification of Aflatoxin B1 by Competitive ELISA

Aflatoxin B1 concentrations in maize samples were determined using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (AFB1 ELISA Kit, Cat. No. E-TO-E027; Elabscience Bionovation, China), following the manufacturer's instructions.

All reagents and samples were equilibrated to room temperature (20–25°C) before analysis. Reagents, including the extraction solution, sample diluent, wash buffer, and horseradish peroxidase (HRP) conjugate, were prepared according to the manufacturer's protocol.

For sample extraction, maize grains were thoroughly homogenized using a high-speed grinder. Weighed approximately 1.00 ± 0.05 g of ground maize into a 15-mL centrifuge tube and added 5 mL of the prepared extraction solution. The mixture was vortexed for 1 minutes and centrifuged at 4,000 rpm for 5 minutes at room temperature.

A 100-μL aliquot of the resulting supernatant was transferred to a clean tube containing 400 μL of Sample Diluent C and mixed thoroughly. Subsequently, 50 μL of the diluted extract was used for ELISA analysis. The reported overall dilution factor was 30.

The assay was performed using a 96-well microplate. Aliquots of 50 μL of AFB1 reference standards, ranging from 0 to 100 ppb, and diluted sample extracts were dispensed into duplicate wells. Subsequently, 50 μL of diluted HRP conjugate and 50 μL of antibody working solution were added to each well.

The microplate was gently mixed for 10 s, sealed, and incubated at 25°C for 30 minutes. Following incubation, the wells were washed four times with 260 μL of wash buffer per well, allowing approximately 30 s between washes to remove unbound reagents.

Next, 100 μL of substrate reagent was added to each well, and the plate was gently mixed for 10 s. The reaction was allowed to proceed in the dark at 25°C for 15 minutes. The enzymatic reaction was terminated by adding 50 μL of stop solution to each well, followed by gentle mixing for 10 s.

Absorbance was measured at 450 nm within 5 minutes of adding the stop solution using a microplate spectrophotometer (Infinite M200 PRO; Tecan, Switzerland).

AFB1 concentrations were calculated from a calibration curve generated using the kit-supplied reference standards and the manufacturer's calculation spreadsheet. The reported overall sample dilution factor of 30 was incorporated into the calculation of AFB1 concentrations in the original maize samples.

Samples producing responses outside the calibration range were reanalyzed after further dilution, using reported dilution factors of 60 or 90, as appropriate. All samples were analyzed in duplicate, and AFB1 concentrations were expressed as μg/kg of maize.

Measured concentrations were subsequently evaluated against the applicable regulatory or reference limits for aflatoxin contamination in maize and animal feed.

2.8. Statistical Analysis

All statistical analyses were performed using IBM SPSS statistics (version 27.0; IBM Corporation, USA) and

Python 3.8 (with SciPy and Pandas libraries). Statistical significance was defined as $P < 0.05$ (two-tailed). Results are presented with 95% confidence intervals where appropriate.

3. RESULTS

3.1. AFB1 contamination Prevalence

Of 120 maize samples examined, 28 samples (23.3%; 95% CI: 16.0-31.5%) exceeded the international regulatory safety limit of 5.0 $\mu\text{g/kg}$, while, 92 samples (76.7%) remained below this threshold (Fig. 2A). The overall AFB1 concentrations exhibited marked skewness, with a median of 1.016 $\mu\text{g/kg}$ reflecting the majority of samples with low contamination, whereas the mean is of 7.01 $\mu\text{g/kg}$ was substantially higher due to the extreme value. The maximum recorded AFB1 concentration was 124.3 $\mu\text{g/kg}$, representing severe contamination approximately 35-fold above the regulatory limit (Table 2). AFB1 positive samples were distributed across all 13 sampled upazilas, with 9-50% prevalence (Fig. 3).

Table 2: AFB1 concentration and moisture content in maize samples

Parameter	Season	Mean $\pm$ SD	Mean Total	Median	Median Total	Min	Max	P value
AFB1 Concentration ($\mu\text{g/kg}$)	Summer	12.3 $\pm$ 21.06	7.01	1.88	1.02	0.21	124.3	<0.001
	Winter	1.71 $\pm$ 3.90		0.60		0.16	19.2	
Moisture Content (%)	Summer	14.38 $\pm$ 3.51	12.97	13.3	11.6	10.2	21.8	<0.001
	Winter	11.56 $\pm$ 1.70		11.6		8.8	17.6	

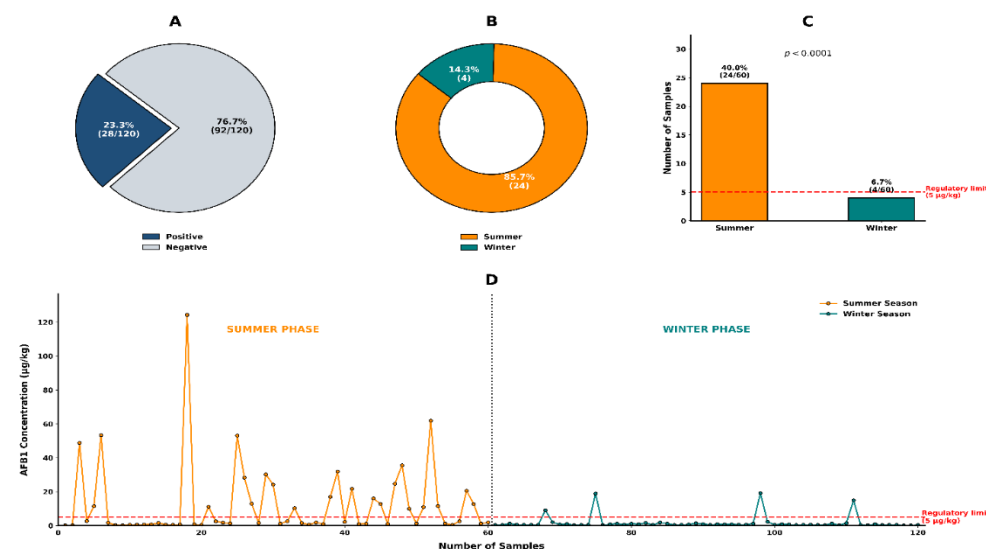


Fig. 2: Prevalence and seasonal distribution of AFB1 contamination in maize samples from Dinajpur District, Bangladesh. A) Overall prevalence AFB1 contamination across all samples B) Seasonal variation in AFB1 prevalence C) Proportion of samples exceeding the regulatory limit (red dashed line) by season. D) Distribution of AFB1 concentrations in individual samples, showing extreme contamination spikes during the summer and winter relative to the regulatory threshold (red dashed line).

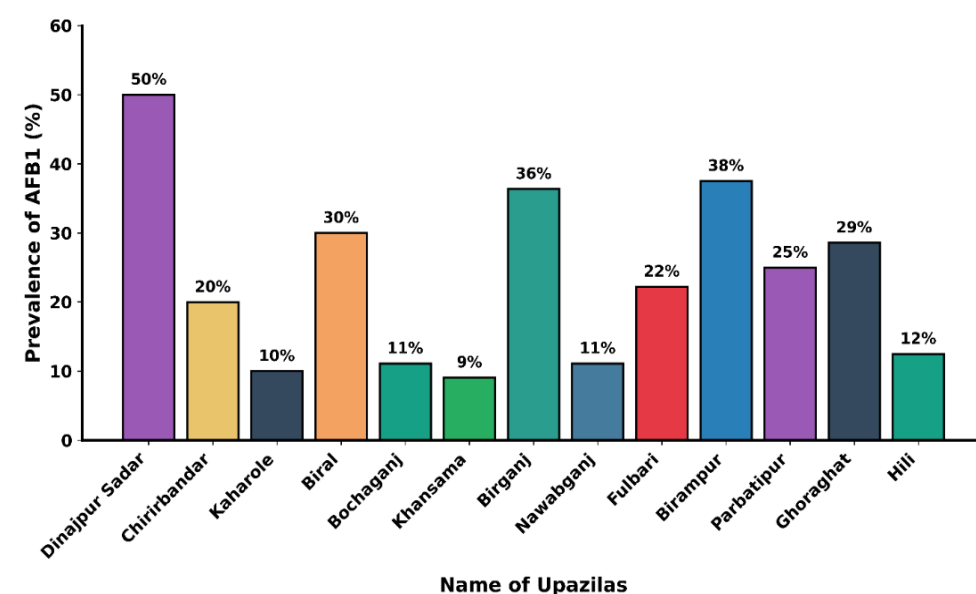


Fig. 3: Geographic variation in AFB1 contamination prevalence across sampled upazilas of Dinajpur District, Bangladesh. Bars represent the percentage of maize samples testing positive for AFB1 in each upazila.

3.2. Seasonal Variation in AFB1 Contamination

AFB1 contamination varied markedly across the two sampling periods. The summer season (June-July) exhibited the highest contamination burden, with 40.0% (95% CI: 28.4-52.6%) of samples exceeding the regulatory limit of 5.0 µg/kg. In contrast, winter (January-February) showed the lowest contamination rate, with only 6.7% (95% CI: 1.4-18.0%) of samples exceeding the limit (Fig. 2C). The vast majority of non-compliant samples (85.7%; 24/28) were collected during the summer season (Fig. 2B and 2D). One-way ANOVA demonstrated highly significant seasonal differences in AFB1 concentration ($P < 0.0001$). Tukey's HSD post hoc test showed that summer samples had significantly higher AFB1 concentrations than winter samples (mean difference = 12.77 µg/kg; 95% CI: 2.91-22.62 µg/kg; $P = 0.008$). Chi-square analysis confirmed that the proportions of non-compliant samples differed significantly by season ($\chi^2 = 17.24$; $P < 0.0001$).

3.3. Grain Moisture Content Variation

Seasonal variation in grain moisture content was substantial. Summer samples exhibited significantly higher moisture content (mean = 14.38; SD = 3.51; median = 13.3; range = 10.2-21.8) compared to winter samples (mean = 11.56; SD = 1.70; median = 11.6; range = 8.8-17.6). One-way ANOVA confirmed significant seasonal differences in moisture content ($P < 0.001$) (Table 2).

3.4. Association Between Moisture Content and AFB1 Contamination

Stratification by moisture content categories revealed a strong dose-response relationship with AFB1 contamination. Samples were categorized into three moisture classes: $\leq 12.0\%$ ($n = 45$), 12.0–14.9% ($n = 50$), and $\geq 15.0\%$ ($n = 25$). The prevalence of samples exceeding the 5.0 µg/kg regulatory threshold increased exponentially with moisture content: only 4.5% (2/45) of samples with $\leq 12.0\%$ moisture were non-compliant, compared to 20.0% (10/50) in the 12.0-14.9% category and 71.4% (17/25) in the $\geq 15.0\%$ category ($\chi^2 = 32.18$; $P < 0.001$). Mean AFB1 concentrations also increased markedly across moisture categories: $\leq 12.0\%$ moisture (mean = 1.203 µg/kg), 12.0-14.9% moisture (mean = 3.871 µg/kg), and $\geq 15.0\%$ moisture (mean = 19.524 µg/kg), representing a 16.2-fold increase from the lowest to highest moisture category. Log-transformed AFB1 concentrations showed a strong positive linear correlation with grain moisture (Pearson $r = 0.672$; $P < 0.001$) (Fig. 4 A-B).

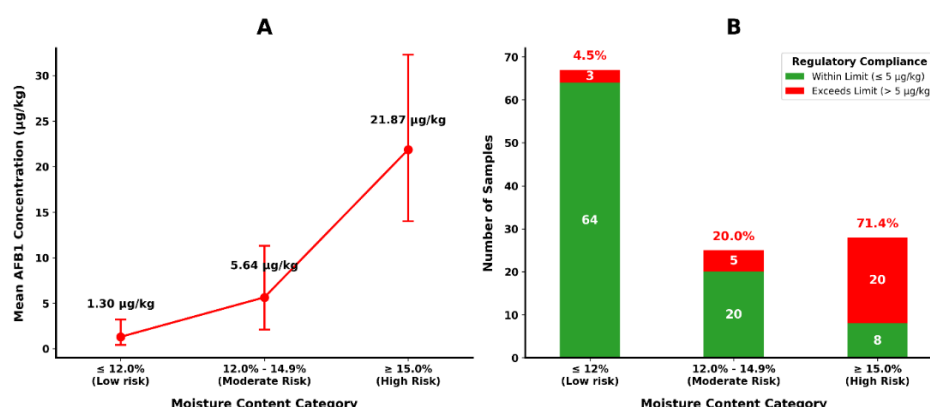


Fig. 4: Influence of moisture content on AFB1 accumulation and regulatory compliance in feed maize. (A) Mean AFB1 concentrations across moisture categories. Data points represent group means. (B) Prevalence of samples conforming to or exceeding the international regulatory limit (5 µg/kg) across the three moisture groups. Numerical annotations within the bars indicate the absolute number of samples, and percentages denote the proportion of non-compliant samples per category.

3.5. Multivariable Analysis of Post-Harvest Risk Factors

Bivariate logistic regression analysis identified moisture content $> 15\%$, prolonged storage duration (3-6 months and > 6 months), visible insect infestation, poor storage ventilation, and storage in a tin shed as significant risk factors for AFB1 non-compliance (Table 3). However, when these variables simultaneously entered into a multivariable logistic regression model using forward stepwise selection, only moisture content and insect infestation remained independent predictors of AFB1 contamination above the regulatory limit (Table 3).

Grain moisture content $> 15\%$ was the strongest independent risk factor, with samples in this category having 22.32-fold higher odds of exceeding the regulatory limit than samples with moisture $\leq 15\%$ (adjusted OR [aOR] = 22.32; 95% CI: 5.18-96.17; $P < 0.001$). Visible insect infestation (classified as mild, moderate, or severe) increased the odds of non-compliance by 5.33-fold compared to samples with no evidence of infestation (aOR = 5.33; 95% CI: 1.45-19.58; $P = 0.012$).

In contrast, although extended storage duration showed a strong crude association in the unadjusted model (COR = 29.75; 95% CI: 5.25-168.45; $P < 0.001$), this association was substantially attenuated after adjustment for moisture content and insect infestation (aOR = 5.11; 95% CI: 0.42-62.38; $P = 0.202$), suggesting that the apparent effect of storage duration was mediated by moisture accumulation and pest-related damage during prolonged storage (Table

3). Similarly, poor storage ventilation was a significant predictor in the crude model (COR = 11.82; 95% CI: 2.82-49.53; P=0.002) but was non-significant after multivariable adjustment (aOR = 0.98; 95% CI: 0.15-6.62; P=0.986), indicating confounding by moisture content.

Table 3: Multivariable Logistic Regression Analysis of Risk Factors for AFB1 Non-Compliance (>5.0 µg/kg)

Risk Factor	Category	n (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Moisture Content	≤15.0%	95 (79.2)	Ref	—	Ref	—
	>15.0%	25 (20.8)	29.75 (5.25–168.45)	<0.001	22.32 (5.18–96.17)	<0.001
Insect Infestation	Absent	85 (70.8)	Ref	—	Ref	—
	Present	35 (29.2)	6.72 (2.18–20.74)	0.001	5.33 (1.45–19.58)	0.012
Storage Duration	<3 months	65 (54.2)	Ref	—	Ref	—
	3–6 months	38 (31.7)	12.67 (2.32–69.23)	0.003	4.88 (0.40–59.47)	0.213
	>6 months	17 (14.2)	29.75 (5.25–168.45)	<0.001	5.11 (0.42–62.38)	0.202
Storage Ventilation	Adequate	42 (35.0)	Ref	—	Ref	—
	Partial	56 (46.7)	4.22 (1.14–15.60)	0.031	2.11 (0.34–13.09)	0.415
	Poor	22 (18.3)	11.82 (2.82–49.53)	0.002	0.98 (0.15–6.62)	0.986

Regarding physical storage structures, storage in tin shed showed a marginally significant increased risk in the crude model (COR: 4.00, 95% CI: 1.01–15.77, P=0.048), compared to brick-wall storage structures (reference category). However, this association was not statistically significant in the adjusted model (data not shown). Storage within the farm (COR: 4.00, 95% CI: 0.93–17.11, P=0.062) and in the owner's house (COR: 2.25, 95% CI: 0.48–10.53, P=0.303) showed non-significant associations with AFB1 non-compliance.

3.6. General Fungal Contamination

Of the 120 maize samples examined, 103 samples (85.8%) demonstrated fungal growth on SDA, after 5 days of incubation at 30°C, yielding a total fungal burden ranging from 10² to 10⁵ CFU/g. Seventeen (14.2%) were negative for fungal growth on SDA. Of the 103 fungal samples, 74 (61.7% of all samples; 71.8% of fungal-positive samples) exhibited morphological characteristics consistent with aflatoxigenic *Aspergillus* species on AFPA, specifically displaying the characteristic orange-yellow reverse coloration of *A. flavus* and *A. parasiticus*.

3.7. Phenotypic and Microscopic Characterization of Isolates

The fungal isolates showed distinct cultural and microscopic features. On SDA, colonies displayed dense mycelial growth with variable pigmentation ranging from pale to dark green. Subculturing on PDA revealed different colony morphologies, including cottony to velvety textures and color variations from cream to dark brown. On AFPA medium, typical isolates of aflatoxigenic *Aspergillus* species exhibited orange/yellow reverse color and a limited growth pattern, allowing conidiophore structures with distinct conidial chains typical of *Aspergillus* spp., and conidial diameters of 3–8 µm. Representative isolate demonstrating diagnostic morphological features are shown in Fig. 5A–D.

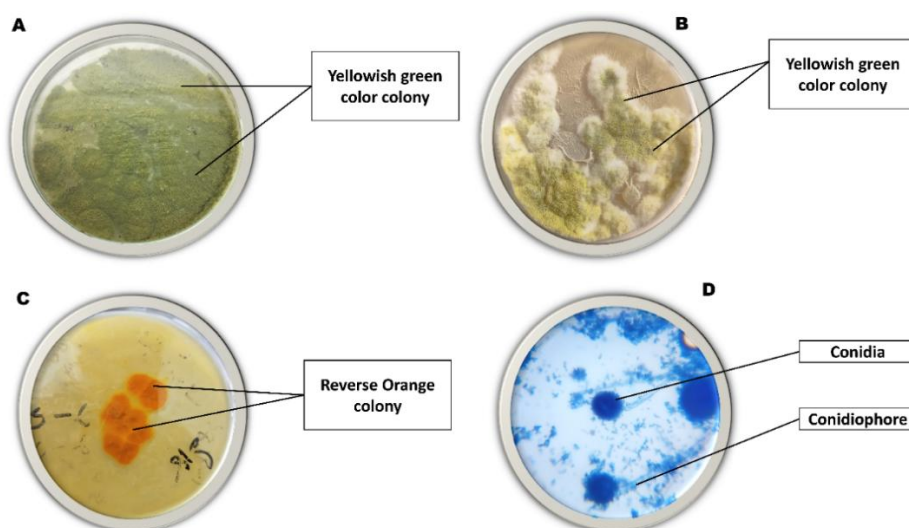
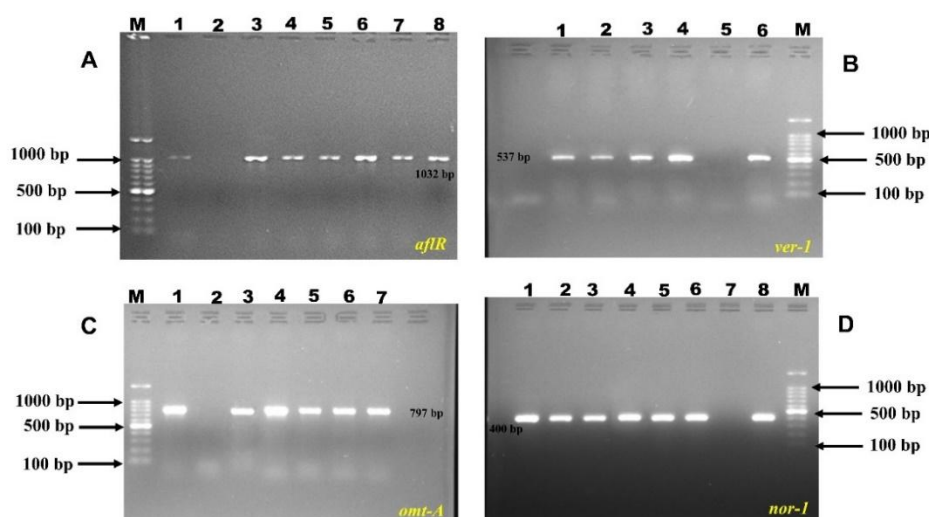


Fig. 5: Cultural and microscopic characterization of fungal isolates recovered from maize samples. (A) Fungal growth on Sabouraud Dextrose Agar (SDA). (B) Colony morphology on Potato Dextrose Agar (PDA). (C) Orange reverse pigmentation on *Aspergillus flavus* and *parasiticus* agar (AFPA), indicative of *A. flavus*/*A. parasiticus* and (D) Microscopic morphology following Lacto phenol Cotton Blue (LPCB) staining, showing characteristic conidiophores and conidia.

3.8. Molecular Detection of Aflatoxin Biosynthetic Genes

DNA extracted on 120 samples was subjected to PCR amplification of four major genes of the aflatoxin biosynthetic pathway (*afIR*, *omt-A*, *nor-I* and *ver-I*). Of these, 47 samples (39.2%) produced no PCR products for any target gene (Supplementary Fig. 1A-D), indicating the absence of aflatoxigenic *Aspergillus* strains or the presence of non-toxigenic variants. PCR amplicons of one or more target genes were obtained in 73 samples (60.8%), suggesting that these strains have the genetic potential to synthesize aflatoxins.



Supplementary Fig. 1: PCR amplification of aflatoxin biosynthetic pathway-associated gene. Representative agarose gel electrophoresis profiles showing amplification of (A) *afIR* (1032 bp), (B) *omt-A* (797 bp), (C) *nor-I* (400 bp), and (D) *ver-I* (537 bp).

The individual gene prevalence among the 73 PCR-positive samples was: *afIR* (71.2%; 52/73), *ver-I* (61.6%; 45/73), *omt-A* (60.3%, 44/73), and *nor-I* (58.9%, 43/73) (Fig. 6). The *afIR* regulatory gene was the most common, with almost three-quarter of toxigenic isolates, which is in line with its vital nature as a master regulator of the aflatoxin biosynthetic pathway.

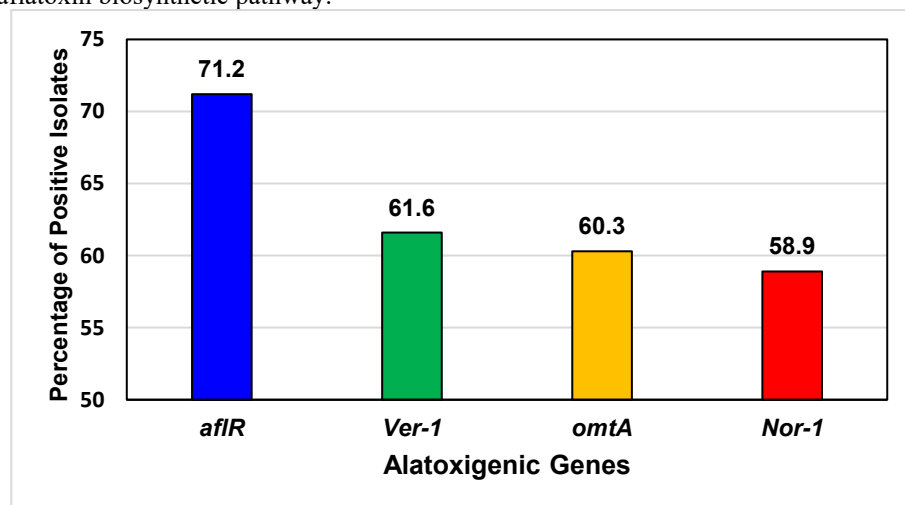


Fig. 6: Frequency of aflatoxigenic genes among AFB1-positive fungal isolates. Horizontal bars show the detection percentage of positive isolates *afIR*, *ver-I*, *omt-A* and *nor-I* genes.

3.9. Multilocus Gene Profiles and Toxigenic Competence Classification

Multilocus gene combinations of the 73 PCR-positive isolates showed extensive genetic heterogeneity in toxigenic potential. Only 11 isolates (15.1%) had single-gene profiles: *omt-A* alone ($n = 7$; 9.6%), *nor-I* alone ($n = 3$; 4.1%), and *afIR* alone ($n = 1$; 1.4%). The predominance of multi-gene combinations indicated high genetic capacity for aflatoxin biosynthesis across the sampled isolates.

The most frequent multi-gene combinations were: *afIR* + *nor-I* + *ver-I* ($n=10$; 13.7%) and *afIR* + *omt-A* + *ver-I* ($n=8$; 11.0%). Strikingly, 12 isolates (16.4%) harbored all four of the target genes (*afIR* + *omt-A* + *nor-I* + *ver-I*), representing the fully competent genotype with the highest toxigenic potential (Fig. 7). These fully competent isolates were disproportionately recovered from samples exceeding the regulatory AFB1 limit: 10 of the 12 four-gene isolates (83.3%) were isolated from non-compliant samples, whereas only 2 (16.7%) originated from compliant samples ($\chi^2 = 14.27$; $P < 0.001$).

4. DISCUSSION

This study documented that 23.3% of maize samples from Dinajpur district exceeded the international regulatory safety limit of 5.0 µg/kg, with concentrations reaching 124.297 µg/kg in severely contaminated samples. The skewed distribution of AFB1 concentrations—characterized by a low median (1.016 µg/kg) but elevated mean (7.006 µg/kg) attributable to extreme outliers – is consistent with mycotoxin contamination patterns documented across South Asia and Sub-Saharan Africa, reflecting the episodic nature of the aflatoxigenic fungal proliferation in agricultural systems (Shabeer et al., 2022; Yu & Pedrosa, 2023). Similar distributions have been reported from India, where 40.2% of maize samples exceeded regulatory limits with maximum concentrations of 1498.32 µg/kg (Muthusamy et al., 2013), and from multiple regions of Bangladesh, where prior studies documented 33-68% non-compliance rates depending on sampling season and region (Lubna et al., 2018; Tarannum et al., 2020). The substantial geographic and temporal heterogeneity in AFB1 distribution suggests contamination occurs through localized, high-risk supply chain nodes rather than uniformly across all maize sources, consistent with prior characterization of Bangladesh's decentralized, small-scale feed production network (Neogi et al., 2024).

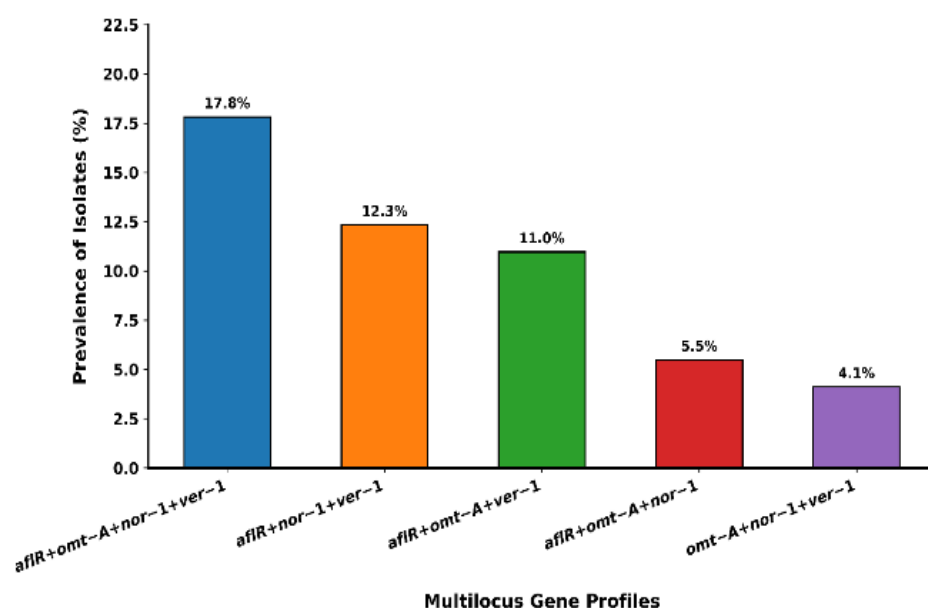


Fig. 7: Multi-locus gene profiles AFB1-positive fungal isolates. Horizontal bars show the detection percentage of isolates carrying each multi-locus combination: *afIR+omt-A+nor-1+ver-1* (all four genes), *afIR+nor-1+ver-1*, *afIR+omt-A+ver-1*, *afIR+omt-A+nor-1*, *omt-A+nor-1+ver-1*.

The significant seasonal difference in AFB1 contamination of this study—40.0% of summer samples contained above the regulatory limit versus 6.7% of winter samples—indicates the key determinants of *Aspergillus* growth and aflatoxin biosynthesis. Summer AFB1 samples (13.87 µg/kg) were 12.6 times higher than winter samples (1.104 µg/kg), which is a difference that is consistent with the extreme seasonal climatic change in Dinajpur, where temperatures are 28^o-32^oC in the summer months, and relative humidity exceeds 75%, and 15^oC and 35% humidity in the winter months, respectively. Mechanistic reasons behind the summer peak of contamination include optimal temperature and humidity for *A. flavus*; *in vitro* experiments showed that optimum toxin production was observed at 25-35°C and a relative humidity >70%, which is exactly the microclimate of the Dinajpur summer (Sugri et al., 2015; Molnár et al., 2023).

The seasonal trends are recorded in South Asian areas. Akbar et al. (2020) observed 95% non-compliance during monsoon months in Punjab, Pakistan, compared with much lower levels in other seasons. Joshi et al. (2022) found that AFB1 concentrations increased 2–3 times in stored maize during monsoon seasons in Northern India. These results contrast with the year-round, high-level contamination observed in certain areas of Sub-Saharan Africa; Akullo et al. (2023) reported 81% non-compliance in Ugandan samples across all seasons, and Akinmusire et al. (2019) reported 73% contamination rates in Nigerian poultry feeds regardless of season. This disparity implies that, although South Asian monsoon systems generate specific temporal windows of risk, the subtropical climate and agricultural infrastructure in East Africa allow the persistent of high-risk conditions throughout the year. The AFB1 contamination in Bangladesh suppressed in winter due to minutesimum microclimate conditions in the area to support fungal secondary metabolism; cold temperatures and humidity induce fungal dormancy and inhibit complex enzyme cascades of aflatoxin biosynthesis that effectively create a natural seasonal control mechanism (Magan & Aldred, 2007; Bradford et al., 2020)

In multivariable analysis, grain moisture content was the most significant independent predictor of AFB1

contamination, with samples containing higher moisture ($>15\%$) having odds 22.32 times higher than samples with lower moisture ($\leq 15\%$). The dose-response relationship was also impressive: only 4.5% of samples with $\leq 12\%$ moisture were above the limit, 20.0% of samples with 12.0–14.9% moisture, and 71.4% of samples with $\geq 15.0\%$ moisture. This exponential relationship is based on basic mycological principles. Moisture content of $\leq 12\%$ corresponds to water activity (a_w) of <0.7 , which is lower than the minimum water activity required to allow growth of even xerophilic *Aspergillus* species; water activity of 14.0–15% raises a_w to 0.85–0.90, which allows rapid spore germination and logarithmic fungal growth (Chauhan et al., 2008; Ali, 2010). At these high moisture levels, the grain microenvironment provides enough available water to support fungal biomass growth and activate the complex regulatory cascades of aflatoxin biosynthesis (Gallo et al., 2016).

The essential role of moisture is highlighted by the fact that long storage periods (>6 months) were significantly correlated with AFB1 contamination but were not significant when moisture content was taken into account. This confounding trend suggests that storage length alone does not drive toxin accumulation; instead, insufficient moisture regulation over time allows gradual absorption and creates conditions favorable to fungal growth. This interpretation is supported by Zheng et al. (2024) and Nakavuma et al. (2020), which show that hermetically sealed storage keeping moisture levels $<12\%$ does not cause accumulation of AFB1 after 12–18 months of storage, but conventional open storage equilibrates moisture and synthesizes aflatoxin in abundance within 30–60 days.

We identified insect infestation (mild, moderate, or severe) as a visible risk factor, and infested samples had 5.33-fold higher odds of regulatory non-compliance. This observation mechanistically indicates two synergistic pathways by which insects enable AFB1 accumulation. First, insect boring directly wounds grain kernels, rupturing the protective seed coat and allowing rapid colonization by *A. flavus* spores that unbroken plant defenses would prevent (Danso et al., 2019; Manu et al., 2019). Second, the active population of insects produces a lot of metabolic heat and moisture during respiration; in the storage masses of grains, it forms local microclimatic conditions of high temperature (35°C above ambient) and moisture (10–15 % ambient), which will encourage the growth of fungi in grain directly outside the feeding spots (Likhayo et al., 2018). These microenvironmental changes are especially problematic in conventional open storage systems. In the crude model, the association between storage ventilation and AFB1 contamination was completely confounded by moisture content and became non-significant after adjustment, suggesting that ventilation's protective effects, despite apparent moisture-control mechanisms, are not due to direct antifungal effects.

Molecular profiling by PCR amplification of four major genes in the aflatoxin biosynthetic pathway (*afIR*, *omt-A*, *nor-1*, *ver-1*) identified 73 isolates (60.8% of all samples) containing genetic components of the aflatoxin operon. The most common single marker was the regulatory gene *afIR* (71.2% of positive isolates). This high frequency is biologically important because *afIR* encodes a master regulatory transcription factor that regulates expression of the 25 genes in the aflatoxin biosynthetic cluster (Criseo et al., 2001; Syamsu et al., 2026b). The absence of a functional *afIR* prevents expression of downstream structural genes (*nor-1*, *ver-1*, *omt-A*) and toxin biosynthesis; therefore, *afIR* prevalence indicates the percentage of isolates with genetic competency for toxin production (Wang et al., 2022).

The striking and mechanistically significant prevalence of multi-gene combinations among isolates was notable. Single-gene profiles were uncommon (15.1% of positive isolates), and only 1 isolate (1.4%) had *afIR* alone and 7 isolates (9.6%) had *omt-A* alone. In contrast, 52 isolates (71.2%) harbored multi-combinations, including 12 isolates (16.4% of all samples; 10.0% of all tested samples) with the complete four-gene suite (*afIR* + *omt-A* + *nor-1* + *ver-1*). These complete isolates have the highest toxigenic potential, with all the necessary biosynthetic enzymes available in intact genomic organization. Comparative molecular analysis supports that the optimum toxin production capacity is only reached when the isolates contain the entire gene set since single structural genes or sub sets of gene produce incomplete cascades and significantly lower toxin production rates (Muthusamy et al., 2013).

Importantly, 83.3% (10/12) of the four-gene isolates were obtained from samples that exceeded the AFB1 limit, and only 16.7% were obtained from compliant samples, indicating a strong correlation between molecular toxigenic competence and real aflatoxin accumulation. By season, 75% (9/12) of fully competent isolates were collected in summer, versus 0 in winter. This seasonal clustering of high-competency strains provides a mechanistic link between molecular genetics and the observed seasonal patterns of contamination.

The key finding of this study is that it demonstrates a highly important gene-environment interaction in defining the severity of AFB1 contamination. The genetic capacity to produce toxins lies in the presence of toxigenic *Aspergillus* strains with high-competency gene profiles (especially the four-gene genotype), although this potential remains dormant unless environmental conditions activate the regulatory and biosynthetic cascades. The environmental epidemiological evidence shows that the two triggers are high moisture content of grains ($>15\%$), and warm temperature ($>28^\circ\text{C}$), and the catalytic cofactor is the microenvironmental modification by insects. The interaction can be theorized as follows: Under winter conditions (low temperature, $<35\%$ RH), even the most toxigenic strains show little metabolic activity, and the *afIR* regulatory gene and, subsequently, structural genes are expressed at the lowest levels. Gene expression increases substantially when ambient temperature rises above 25°C ;

however, toxin production remains limited if grain moisture is <12% because low water activity prevents both fungal biomass accumulation and the osmotic conditions necessary for secondary metabolism (Ali et al., 2022). When summer conditions coincide with grain moisture >15%, optimal temperature, high water activity, and active insect infestation create the perfect “genomic-environmental storm,” in which high-competency strains activate their full biosynthetic machinery, resulting in rapid, exponential AFB1 synthesis. This explains the 12.6-fold difference in mean AFB1 concentrations between summer and winter, and the concentration of severe outliers (>50 µg/kg) exclusively within summer, high-moisture, insect-infested samples (Zanon et al., 2022; Stutt et al., 2023).

Multiple sources support this gene-environment interaction. First, the temporal clustering of fully competent isolates in the summer sample showed that strain selection is environment-dependent, summer conditions may actively select for or enable detection of high-competency strains through increased spore production. Second, the exponential dose-response relationship between moisture and contamination (4.5% compliance at ≤12% moisture versus 71.4% non-compliance at >15% moisture) could only occur if the underlying genetic toxigenic potential exists but is conditionally expressed based on environmental signals. Third, the observation that storage duration per se is not an independent risk factor, only exerting effects through moisture accumulation, indicates that prolonged storage activated gene expression through a moisture-mediated pathway rather than through time-dependent mechanisms.

The implications of this model of gene-environment interaction on risk-based mitigation strategies are significant. Environmental interventions, such as keeping the moisture level <12% and limiting the infestation of insects, would be effective in silencing the aflatoxin biosynthetic genes of even highly toxigenic strains and preventing AFB1 synthesis regardless of genetic competency. On the other hand, strain substitution with atoxigenic *A. flavus* strains (biological control), without environmental control, would likely be ineffective in high-risk seasons when native, toxigenic strains are more competitive (Wanjiru et al., 2023).

This research provides vital evidence supporting environment-based mitigation measures for the poultry feed industry in Bangladesh. Because only moisture content and insect infestation are independent, non-confounded risk factors, introducing interventions to control these two variables could substantially reduce the risk of AFB1 contamination and regulatory non-compliance. The observation of a unique summer period of increased risk (May–September) allows targeting prevention measures, intensive drying, storage surveillance, and pest control during peak risk seasons, potentially reaching compliance levels similar to those seen in winter (>90% compliance at current moisture levels). This finding is in line with that of Khan et al. (2005), which has indicated the highest incidence of aflatoxins was observed in June–August and high moisture–aflatoxin relationship ($r = 0.814$) in Bangladeshi poultry feed. For public health, AFB1 bioaccumulation in poultry tissues and eggs could provide a direct route of human exposure; even a small decrease in feed contamination would significantly reduce the dietary burden, especially for vulnerable groups in Bangladesh and South Asia.

4.1. Limitations of the Study

First, the cross-sectional, one-district sampling design does not allow generalization to the poultry feed supply chain across Bangladesh, which has several agroecological zones with unique climate, agronomy, and infrastructure capacity. The samples were taken during one annual cycle; a longer-term observation over several years would better capture inter-annual variability driven by rainfall patterns and other climatic variations. Second, molecular profiling detected biosynthetic genes at the DNA level by PCR but did not quantify gene expression (transcriptomics via RT-qPCR) or fungal biomass (via qPCR). Consequently, we cannot determine whether the presence of four-gene isolates reflects constitutive expression of the entire biosynthetic pathway or merely genetic potential with conditional expression. Third, toxicological assessment was limited to AFB1; poultry and livestock may be exposed to multiple mycotoxins simultaneously, and fumonisin (FUM), deoxynivalenol (DON) (Wang et al., 2021), and other *Fusarium* toxins frequently co-occur with AFB1 in stored grains, potentially creating synergistic or antagonistic toxicological effects not captured by single-toxin assessment. Fourth, the sample size of 120 specimens, while adequate for univariable epidemiological assessment, may be underpowered to detect interactions between environmental variables and for subgroup analyses stratified by region or commodity type.

5. CONCLUSION

This cross-sectional study found aflatoxin B1 (AFB1) contamination in 23.3% of maize samples collected from Dinajpur District, Bangladesh, with a higher proportion exceeding the applied regulatory threshold during summer (40.0%). Grain moisture content above 15.0% and visible insect infestation were identified as independent factors associated with AFB1 non-compliance, with elevated moisture content showing the strongest association. Molecular analysis revealed that 60.8% of samples contained *Aspergillus* isolates carrying one or more aflatoxin biosynthetic genes, while 16.4% harbored isolates positive for all four targeted genes (*aflR*, *omtA*, *nor-1*, and *ver-1*). Notably, 83.3% of isolates carrying all four genes originated from non-compliant samples, and 75.0% were recovered during summer. These findings suggest that the presence of aflatoxin biosynthetic genes, along with unfavorable post-harvest

conditions, may increase contamination risk. However, the presence of these genes does not independently confirm their expression or active aflatoxin production. The results highlight the importance of modifiable post-harvest factors, particularly grain moisture and insect infestation, in managing AFB1 contamination. Therefore, prioritize rapid post-harvest drying, maintaining appropriately low moisture levels during storage and distribution, improved ventilation, and integrated pest management as practical preventive measures. Maintaining maize moisture at or below 12% may be a useful storage-management target, although its effectiveness depends on temperature, water activity, storage duration, and other environmental conditions. Overall, the findings provide location-specific evidence supporting integrated post-harvest management and targeted AFB1 surveillance to improve maize quality and safety within Bangladesh's poultry feed supply chain.

Declarations

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Data Availability: All data supporting the study's findings are included in the article.

Ethics Statement: This study did not involve live animals or human subjects directly. The research used maize samples from a commercial farm, feed mill, and retail store; therefore, ethical approval was not required.

Author's Contributions: M.A.H. contributed to the conceptualization, formal analysis, supervision, project administration, and writing of the original draft. A.H. contributed to the methodology, investigation and data collection, laboratory analysis, and visualization. M.S.S.S. contributed to the methodology, investigation and data collection, and visualization. H.M. contributed to the investigation and data collection, formal analysis, and laboratory analysis. S.P. contributed to the literature review, manuscript review, and editing. J.A.S. contributed to manuscript review, editing, and finalization. All authors contributed to the review and editing of the manuscript and have read and agreed to the published version of the manuscript.

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REFERENCES

- Abbas, R. Z., Raketsky, V., Munir, F., Mustafin, B., & Aubakirov, M. (2024). Botanicals in ameliorating mycotoxicosis in poultry. *International Journal of Veterinary Science*, 13(6), 878–888. <https://doi.org/10.47278/journal.ijvs/2024.232>
- Akbar, N., Nasir, M., Naeem, N., Ahmad, M. D., Saeed, F., Anjum, F. M., Iqbal, S., Tufail, T., Shah, F. H., & Atif, M. (2020). Assessment of aflatoxin in milk and feed samples and impact of seasonal variations in the Punjab, Pakistan. *Food Science & Nutrition*, 8(6), 2699–2709. <https://doi.org/10.1002/fsn3.1557>
- Akinmusire, O. O., El-Yuguda, A.-D., Musa, J. A., Oyedele, O. A., Sulyok, M., Somorin, Y. M., Ezekiel, C. N., & Krska, R. (2019). Mycotoxins in poultry feed and feed ingredients in Nigeria. *Mycotoxin Research*, 35(2), 149–155. <https://doi.org/10.1007/s12550-018-0337-y>

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- Akullo, J. O., Okello, D. K., Mohammed, A., Amayo, R., Okello, D. K., Moyinda, R., Magumba, D., Gidoi, R., & Mweetwa, A. M. (2023). Aflatoxin contamination in groundnut and maize food products in Eastern and Northern Uganda. *Cogent Food & Agriculture*, 9(1), 2221015. <https://doi.org/10.1080/23311932.2023.2221015>
- Ali, A. M. (2010). Studying the effect of moisture content and temperature of corn stored in ware-houses and out-doors for production and determination of Aflatoxin B1. *Journal of Wasit for Science and Medicine*, 3(1), 58-66. <https://doi.org/10.31185/jwsm.98>
- Ali, M. Z., Sana, S., Sheikh, A. A., & Maheen, Z. (2022). Molecular characterization of toxigenic aspergillus flavus isolated from sick broiler lungs and risk factors analysis. *Pakistan Veterinary Journal*, 42(2), 194–200. <https://doi.org/10.29261/pakvetj/2022.037>
- Bereda, G. (2026). Aflatoxin contamination in maize: Food safety challenges, analytical advances, exposure assessment, and mitigation approaches. *Journal of Food Composition and Analysis*, 158, 109443. <https://doi.org/10.1016/j.jfca.2026.109443>
- Bradford, K., Dahal, P., Asbrouck, J. V., Kunusoth, K., Bello, P., Thompson, J. F., & Wu, F. (2020). The dry chain: reducing postharvest losses and improving food safety in humid climates. *Trends in Food Science and Technology*, 71, 84-93. <https://doi.org/10.1016/j.tifs.2017.11.002>
- Caceres, I., Al Khoury, A., El Khoury, R., Lorber, S., P. Oswald, I., El Khoury, A., Atoui, A., Puel, O., & Bailly, J.-D. (2020). Aflatoxin biosynthesis and genetic regulation: a review. *Toxins*, 12(3), 150. <https://doi.org/10.3390/toxins12030150>
- Chauhan, Y., Wright, G., & Rachaputi, N. (2008). Modelling climatic risks of aflatoxin contamination in maize. *Australian Journal of Experimental Agriculture*, 48(3), 358-366. <https://doi.org/10.1071/ea06101>
- Chupia, V., Tangtrongsup, S., Saedan, A., Ounnunkad, J., Pikulkaew, S., Suriyasathaporn, W., & Chaisri, W. (2022). Impact of storage conditions and mold types on Aflatoxin B1 concentration in corn residue used as dairy feed in small holder dairy farms, Thailand. *Biocontrol Science*, 27(2), 99-105. <https://doi.org/10.3480/biocontrolscience.27.99>
- Criseo, G., Bagnara, A., & Bisignano, G. (2001). Differentiation of aflatoxin-producing and non-producing strains of *Aspergillus flavus* group. *Letters in Applied Microbiology*, 33(4), 291–295. <https://doi.org/10.1046/j.1472-765X.2001.00998.x>
- Danso, J. K., Osekere, E. A., Opit, G. P., Arthur, F. H., Campbell, J. F., Mbata, G., Manu, N., Armstrong, P. R., & McNeill, S. G. (2019). Impact of storage structures on moisture content, insect pests and mycotoxin levels of maize in Ghana. *Journal of Stored Products Research*, 81, 114-120. <https://doi.org/10.1016/j.jspr.2018.11.012>
- Fouad, A.M., Ruan, D., El-Senousey, H.K., Chen, W., Jiang, S., Zheng, C. (2019). Harmful effects and control strategies of aflatoxin B1 produced by *Aspergillus flavus* and *Aspergillus parasiticus* strains on poultry: review. *Toxins*, 11(3), 176. <https://doi.org/10.3390/toxins11030176>
- Frisvad, J. C., & Larsen, T. O. (2016). Extrolites of *Aspergillus fumigatus* and other pathogenic species in *Aspergillus* section *Fumigati*. *Frontiers in Microbiology*, 6, 1485. <https://doi.org/10.3389/fmicb.2015.01485>
- Gallo, A., Solfrizzo, M., Epifani, F., Panzarini, G., & Perrone, G. (2016). Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *Aspergillus flavus* on almond medium. *International Journal of Food Microbiology*, 217, 162–169. <https://doi.org/10.1016/j.ijfoodmicro.2015.10.026>
- Haque, M.A., Wang, Y., Shen, Z., Li, X., Saleemi, M.K., & He, C. (2020). Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review. *Microbial Pathogenesis*, 142, 104095. <https://doi.org/10.1016/j.micpath.2020.104095>
- Haris, R. S. W., Purwanti, S., & Syamsu, J. A. (2026). Effects of coarse and fine ground corn particle size distribution on physical characteristics and nutrient digestibility of layer diets. *Agrobiological Records*, 25, 70–78. <https://doi.org/10.47278/journal.abr/2026.048>
- Hassen, J. Y., Debella, A., Eyeberu, A., & Mussa, I. (2024). Level of exposure to aflatoxins during pregnancy and its association with adverse birth outcomes in Africa: a meta-analysis. *International Health*, 16(6), 577–591. <https://doi.org/10.1093/inthealth/ihae015>
- Joshi, P., Chauysrinule, C., Mahakarnchanakul, W., & Maneeboon, T. (2022). Multi-mycotoxin contamination, mold incidence and risk assessment of aflatoxin in maize kernels originating from Nepal. *Microbiological Research*, 13(2), 275-285. <https://doi.org/10.3390/microbiolres13020021>
- Kamal, M., Al-Mamun, M., Hossain, M., Hasan, M., & Hashem, M. (2022). Assessment of aflatoxin in beef cattle feed and feed ingredients in Bangladesh: A safety issue. *Meat Research*, 2(4). <https://doi.org/10.55002/mr.2.4.27>
- Kępka-Borkowska, K., Chałasiński, K., Ogłuszka, M., Borkowski, M., Lepczyński, A., Pareek, C. S., Starzyński, R. R., Lichwiarska, E., Sultana, S., Kalra, G., Purohit, N., Gralak, B., Poławska, E., & Pierzchała, M. (2025). Current approaches to Aflatoxin B1 control in food and feed safety: Detection, inhibition, and mitigation. *International Journal of Molecular Sciences*, 26(13), 06534. <https://doi.org/10.3390/ijms260606534>
- Khan, M. M. H., Chowdhury, B., Bhuiya, M. R. H., & Rahim, M. (2005). Variation of aflatoxin level in different poultry feeds used in different poultry farms of Bangladesh round the year. *International Journal of Poultry Science*, 4(6), 382–387. <https://doi.org/10.3923/ijps.2005.382.387>
- Likhayo, P., Bruce, A., Tefera, T., & Mueke, J. (2018). Maize grain stored in hermetic bags: effect of moisture and pest infestation on grain quality. *Journal of Food Quality*, 2018(1), 2515698. <https://doi.org/10.1155/2018/2515698>
- Lubna, M. A., Debnath, M., & Hossaini, F. (2018). Detection of aflatoxin in poultry feed and feed materials through immuno based assay from different poultry farms and feed factories in bangladesh. *Bangladesh Journal of Microbiology*, 35(1), 75-78. <https://doi.org/10.3329/bjm.v35i1.39807>
- Magan, N., & Aldred, D. (2007). Post-harvest control strategies: minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, 119(1-2), 131-139. <https://doi.org/10.1016/j.ijfoodmicro.2007.07.034>

Citation: Hossain, A., Showrav. M.S.S., Mahmud. H., Purwanti. S., Syamsu. J.A., & Haque, M.A. (2026). Assessment of aflatoxin B1 contamination and risk factors in maize-based poultry feed in Bangladesh. *Agrobiological Records*, 26, 161-177. <https://doi.org/10.47278/journal.abr/2026.091>

- Mannaa, M., & Kim, K. D. (2018). Effect of temperature and relative humidity on growth of *Aspergillus* and *Penicillium* spp. and biocontrol activity of *Pseudomonas protegens* AS15 against aflatoxigenic *Aspergillus flavus* in stored rice grains. *Mycobiology*, 46(3), 287–295. <https://doi.org/10.1080/12298093.2018.1505247>
- Manu, N., Opit, G. P., Osekere, E. A., Arthur, F. H., Mbata, G., Armstrong, P., Danso, J. K., McNeill, S. G., & Campbell, J. F. (2019). Moisture content, insect pest infestation and mycotoxin levels of maize in markets in the northern region of Ghana. *Journal of Stored Products Research*, 80, 10–20. <https://doi.org/10.1016/j.jspr.2018.10.007>
- Mitchell, N. J., Riley, R. T., Egnor, P. A., Groopman, J. D., & Wu, F. (2017). Chronic aflatoxin exposure in children living in Bhaktapur, Nepal: Extension of the MAL-ED study. *Journal of Exposure Science and Environmental Epidemiology*, 27(1), 106–111. <https://doi.org/10.1038/jes.2015.87>
- Molnár, K., Rácz, C., Dövényi-Nagy, T., Bakó, K., Pusztahelyi, T., Kovács, S., Adácsi, C., Pócsi, I., & Dobos, A. (2023). The effect of environmental factors on mould counts and aflatoxin production by *Aspergillus flavus* in maize. *Toxins*, 15(3). <https://doi.org/10.3390/toxins15030227>
- Mongkon, W., Sugita-Konishi, Y., Chaisri, W., & Suriyasathaporn, W. (2017). Aflatoxin B1 contamination of dairy feeds after storage in farm practice in tropical environment. *Biocontrol Science*, 22(1), 41–45. <https://doi.org/10.3480/biocontrolscience.22.41>
- Monson, M.S., Settlege, R.E., McMahon, K.W., Mendoza, K.M., Rawal, S., El-Nezami, H.S., Coulombe, R.A., Reed, K.M. (2014). Response of the hepatic transcriptome to aflatoxin B1 in domestic turkey (*Meleagris gallopavo*). *PLoS One*, 9(6), e100930. <https://doi.org/10.1371/journal.pone.0100930>
- Muthusamy, K., Arumugam, K., Rethinasamy, V., Srinivasan, M., & Thangamuthu, J. (2013). Occurrence of aflatoxin contamination in maize kernels and molecular characterization of the producing organism, *Aspergillus*. *African Journal of Biotechnology*, 12(40), 5839–5844. <https://doi.org/10.5897/AJB12.2366>
- Nakavuma, J. L., Waheru, A., Okello, D. K., Amayo, R., & Okello, D. K. (2020). Assessment of practices, knowledge and risk factors associated with aflatoxin contamination of poultry feeds in Uganda. *International Journal of Food Contamination*, 7(1), 1. <https://doi.org/10.1186/s40550-020-00079-2>
- Neogi, S. B., Uddin, M. N., Arif, M., Şen, A., Paul, P. R., Saddam, M. J. I., & Kabir, S. M. L. (2024). Influence of climatic factors on spatio-temporal variabilities in the occurrence of aflatoxins in major food grains available at retail shops in Bangladesh. *Asian-Australasian Journal of Food Safety and Security*, 8(2), 32–47. <https://doi.org/10.3329/aaajfss.v8i2.72825>
- Nizamiyoglu, F., & Oguz, H. (2003). Occurrence of aflatoxins in layer feed and corn samples in Konya province, Turkey. *Food Additives & Contaminants*, 20(7), 654–658. <https://doi.org/10.1080/02652030303842>
- Peng, X., Bai, S., Ding, X., Zeng, Q., Zhang, K., & Fang, J. (2015). Pathological changes in the immune organs of broiler chickens fed on corn naturally contaminated with aflatoxins B1 and B2. *Avian Pathology*, 44(3), 192–199. <https://doi.org/10.1080/03079457.2015.1023179>
- Saha Turna, N., & Wu, F. (2019). Risk assessment of aflatoxin-related liver cancer in Bangladesh. *Food Additives & Contaminants: Part A*, 36(2), 320–326. <https://doi.org/10.1080/19440049.2019.1567941>
- Sana, S., Anjum, A. A., Yaqub, T., & Maheen, Z. (2025). Contamination of poultry feed with aflatoxin-producing *Aspergillus flavus* poses a serious food safety risk. *Annals of Microbiology*, 75, 36. <https://doi.org/10.1186/s13213-025-01808-8>
- Shabeer, S., Asad, S., Jamal, A., & Ali, A. (2022). Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review. *Toxins*, 14. <https://doi.org/10.3390/toxins14050307>
- Stutt, R., Castle, M. D., Markwell, P., Baker, R., & Gilligan, C. A. (2023). An integrated model for pre- and post-harvest aflatoxin contamination in maize. *NPJ Science of Food*, 7, 60. <https://doi.org/10.1038/s41538-023-00238-7>
- Sugri, I., Osiru, M., Larbi, A., Buah, S. S. J., Nutsugah, S. K., Asieku, Y., & Lamini, S. (2015). Aflatoxin management in Northern Ghana: Current prevalence and priority strategies in maize (*Zea mays* L). *Journal of Stored Products and Postharvest Research*, 6(6), 48–55. <https://doi.org/10.5897/JSPR2015.0184>
- Sultana, S., Rezve, M., Bushra, F., Yeasmin, F., Khan, S. A. K. U., & Abdullah Al Mamun, S. M. (2026). Molecular characterization and aflatoxin biosynthetic gene profiles of *Aspergillus flavus* associated with maize and chili in Bangladesh. *Fungal Biology*, 130(6), 101826.
- Syamsu, J. A., Amal, I., Wijaya, A., Amas, A. F., Walinono, A. M. F. A., Yamin, A. A., & Purwanti, S. (2025a). Optimization of ground corn particle size in the diet to improve physical pellet quality in native chicken feed production. *International Journal of Agriculture and Biosciences*, 14(6), 1438–1445. <https://doi.org/10.47278/journal.ijab/2025.166>
- Syamsu, J. A., Purwanti, S., Yamin, A. A., Amal, I., & Samsudin, A. A. (2025b). Evaluating physical and chemical quality of corn kernel as poultry feed ingredient in the procurement of feed mill raw material. *International Journal of Agriculture and Biosciences*, 14(4), 683–692. <https://doi.org/10.47278/journal.ijab/2025.070>
- Syamsu, J. A., Purwanti, S., Yamin, A. A., Amal, I., Irawan, F., Haque, M. A., & Chung, E. L. T. (2026b). Prediction of aflatoxin risk in corn feed based on physical quality parameters: Development and validation of ordinal models. *Agrobiological Records*, 25, 38–50. <https://doi.org/10.47278/journal.abr/2026.045>
- Syamsu, J.A., Purwanti, S., Yamin, A.A., Amal, I., Haque, M.A., Chung, E.L.T. (2026a). Relationships between physical characteristics and aflatoxin contamination in maize as a poultry feed ingredient. *International Journal of Veterinary Science*, 15(5): 1582–1590. <https://doi.org/10.47278/journal.abr/2026.045>
- Tarannum, N., Nipa, M. N., Das, S., & Parveen, S. (2020). Aflatoxin M1 detection by ELISA in raw and processed milk in Bangladesh. *Toxicology Reports*, 7, 1339–1343. <https://doi.org/10.1016/j.toxrep.2020.09.012>
- Wang, P., Xu, J., Chang, P.-K., Liu, Z., & Kong, Q. (2022). New insights of transcriptional regulator AflR in *Aspergillus flavus* physiology. *Microbiology Spectrum*, 10(1), e00791–21. <https://doi.org/10.1128/spectrum.00791-21>

Citation: Hossain, A., Showrav. M.S.S., Mahmud, H., Purwanti, S., Syamsu, J.A., & Haque, M.A. (2026). Assessment of aflatoxin B1 contamination and risk factors in maize-based poultry feed in Bangladesh. *Agrobiological Records*, 26, 161–177. <https://doi.org/10.47278/journal.abr/2026.091>

- Wang, Y., Quan, H., Li, X., Li, Q., Haque, M. A., Shi, Q., Fu, Q., & He, C. (2021). Contamination with fumonisin B and deoxynivalenol is a threat to egg safety and contributes to gizzard ulcerations of newborn chickens. *Frontiers in Microbiology*, 12, 676671. <https://doi.org/10.3389/fmicb.2021.676671>
- Wanjiru, J. W., Njue, L. G., Okoth, M. W., & Muriira, G. K. (2023). Risk mitigation of aflatoxin contamination in maize and its food and feed products in developing countries: A review. *East African Journal of Science, Technology and Innovation*, 4. <https://doi.org/10.37425/eajsti.v4i.742>
- Wei, Y., Sun, M., Sun, J., Jiang, Q., & Zhang, B. (2024). Effect of dietary supplementation of mycotoxin adsorbent on laying performance and oviduct health of laying hens in aflatoxin B1 exposed. *Agriculture*, 14, 2176. <https://doi.org/10.3390/agriculture14122176>
- Yalew, A. M., Negawo, A. T., Alemu, T., Hanson, J., Blummel, M., Woldeyohannis, N. N., Teferi, Y. A., Asmare, Y., Kodukula, P. V. S. V., & Jones, C. S. (2026). Aflatoxin B1 contamination and fungal diversity in oilseed cakes and formulated feeds from livestock feed markets in Addis Ababa and surrounding areas. *Mycotoxin Research*, 42, 24. <https://doi.org/10.1007/s12550-025-00630-0>
- Yamin, A. A., Ridwan, M., Purwanti, S., & Syamsu, J. A. (2025). Analysis of the sustainability of small-scale feed mills in supporting poultry industry in Sidenreng Rappang Regency. *International Journal of Agriculture and Biosciences*, 14(1), 153–163. <https://doi.org/10.47278/journal.ijab/2024.192>
- Yohannis, E., Urugo, M.M., Tekla, T.A., Getachew, P., Tola, Y.B., Forsido, S.F., Kebede, Y.S., & Teferra, T.F. (2025). Aflatoxin contamination in agri-food systems: a comprehensive review of toxicity, food security, economic impacts, and sustainable mitigation across the value chain. *Food Science and Nutrition*, 13(10), e71104. <https://doi.org/10.1002/fsn3.71104>
- Yu, J., & Pedrosa, I. (2023). Mycotoxins in cereal-based products and their impacts on the health of humans, livestock animals and pets. *Toxins*, 15. <https://doi.org/10.3390/toxins15080480>
- Zanon, M. A. S., Bossa, M., Chiotta, M., Oddino, C., Giovanini, D., Cardoso, M. L., Bartosik, R., & Chulze, S. (2022). Pre-harvest strategy for reducing aflatoxin accumulation during storage of maize in Argentina. *International Journal of Food Microbiology*, 380, 109887. <https://doi.org/10.1016/j.ijfoodmicro.2022.109887>
- Zeweld, S. W., Tarekegn, E. K., & Welearegay, M. A. (2025). Detection of aflatoxigenic fungi in dairy cattle feed: Findings from urban districts of Tigray, Ethiopia. *Veterinary Medicine and Science*, 11, e70362. <https://doi.org/10.1002/vms3.70362>
- Zheng, Y., Wu, W., Sun, C., Liu, H., & Dou, J. (2024). Occurrence and fate analysis of mycotoxins in maize during the post-harvest period. *Toxins*, 16. <https://doi.org/10.3390/toxins16110459>