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Detection of aflatoxigenic fungi and aflatoxin levels in poultry feed

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ABSTRACT

The present research work was carried out for the detection of aflatoxigenic fungus from poultry feed samples. During a period of 12 months (from June 2018 to June 2019), a total of 100 poultry feed samples comprising of 50 commercially prepared and 50 self compounded poultry feed were collected from different 5 different farms of Dinajpur districts, Bangladesh. Among the 100 feed samples *Aspergillus* spp was found on 54 feed samples with 54% prevalence. But Aflatoxigenic *Aspergillus* spp was found on 48 samples with 48% prevalence. In this study the prevalence of *Aspergillus* spp was 56% in self compounded poultry feed and 52% prevalence in commercially prepared poultry feed. There was no significant difference in the prevalence of *Aspergillus* spp and also in the prevalence of aflatoxigenic fungus isolated from commercially prepared feed as well as in self compounded feed on the basis of farms. Maximum level of aflatoxin was ≥20 ppb. This study alarms us about the potential risks of *Aspergillus* spp to public health if contaminate agricultural commodities such as grains or raw materials such as poultry feed.

INTRODUCTION

Nearly every food or feed commodity can be contaminated by fungal pathogens and many of these fungi are capable of producing one or more mycotoxins. Aflatoxins are types of mycotoxins that are produced by certain molds, which grow in soil, decaying vegetation, hay and grains.

A group of secondary metabolites produced by members of *Aspergillus* spp. (commonly by *Aspergillus flavus* and *Aspergillus parasiticus*) is known as aflatoxins (Kuiper-Goodman, 1998). These are ubiquitous in nature, associated with the spoilage and toxin production of stored, agricultural commodities (Hedayati et al., 2007). Mycotoxins are often found as natural contaminants in raw ingredients of poultry feed (Khan et al., 2011). Different mycotoxins have been reported as contaminant of poultry feed, most important of which are aflatoxins (B_1, B_2, G_1, G_2) (Gentles et al., 1999).

Considerable importance is associated with the presence of aflatoxins in food and feed because of their carcinogenic, mutagenic and teratogenic effects (Begum and Samajpati, 2000). Poultry are highly susceptible to mycotoxicoses caused by aflatoxins (Anjum et al., 2011). Aflatoxins are the most studied group of mycotoxins which apart from producing clinical toxicosis also reduce the resistance to diseases and interfere with vaccine induced immunity in poultry birds (Sharma, 1993). In poultry, aflatoxin impairs most of the important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, and male and female reproductive performance (Hussain et al., 2010).

Poultry diets are based on cereals and cereal by-products upto 50-60% on a dry matter basis, and these raw materials are the preferred substrates for Penicillium and *Aspergillus* growth (Petzinger and Weindenbach, 2002). In Bangladesh, different feed ingredients that are used in poultry feeds are likely to be contaminated with aflatoxins

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producing fungi. Because, most commercial feed mills in Bangladesh provide suitable environments for fungal growth provoked by improper harvesting and storage, unhygienic method of processing and production, poor methodology of consumption and utilization.

Practically, it is hard to prevent aflatoxin contamination in feed commodities but several measures could control the severity of this aflatoxicosis problem. Therefore, regular monitoring of aflatoxins in poultry feeds is an important precondition to check toxins buildup in poultry feeds. Earlier detection of aflatoxigenic fungi can be made by simple traditional identifications using macro and micro morphological fungal features rather than adopting the time and cost consuming molecular identification techniques. (Mohammad et al., 2019).

Extensive research work on aflatoxin contamination in poultry feed have been done worldwide, but report on occurrence of toxigenic fungi and level of mycotoxin in different products of Bangladesh is very little. On this context, this study was an effort to early investigate distribution of toxigenic fungi in poultry feeds which can help to take preventive measures to combat economic and health losses.

The present study was undertaken for isolation and identification of aflatoxin producing fungus and also for determination of aflatoxin level in poultry feed samples in Bangladesh.

MATERIALS AND METHODS

Samples collection and transportation

A total of 100 samples (50 commercially prepared feed samples and 50 self-compounded feed samples) were collected from different local farms of Dinajpur district, Bangladesh and brought to the microbiology laboratory, HSTU. Approximately 300 g of each feed samples were collected and kept in an ice-box during transportation to the laboratory and stored at 4°C until testing. They were analyzed within 24 hours of sampling.

Processing of sample

300 gm of different poultry feed samples were uniformly homogenized in mortar and pastle using a sterile diluent as per recommendation of ISO, 1995. A homogenized suspension was made with the help of mortar and pastle. A quantity of 10 ml homogenate samples transferred carefully into a sterile testube containing 90 ml of PBS. Thus 1:10 dilution of the samples was obtained.

Isolation and identification of *Aspergillus* spp.

Culture into different media

With the help of sterile inoculating loop the processed samples were inoculated into the Sabroud Dextrose Agar (SDA) and incubated at 37°C for 5-7 days. Colonis from Sabroud Dextrose Agar were subcultured in Potato Dextrose Agar Media.

Microscopic study

Suspected colonies stained by lactophenol cotton blue (LPCB) were examined microsporically for identification of *Aspergillus* spp. Micromorphological characteristics were observed by wet mount in lactophenol cotton blue stain for identification by the conidiospore appearance and arrangement (Thilagam et al., 2016).

Detection of aflatoxin producing ability

Aspergillus Differential Agar Base (ADAB) was used to detect aflatoxin producing ability of the fungal isolates. Observed specific colonies from Sabroud Dextrose Agar and Potato Dextrose agar were subcultured in *Aspergillus flavus parasiticus* agar medium. A bright orange colour on the reverse side of the plates of *Aspergillus* Differentiation Medium Base will indicate a positive result (Thilagam et al. 2016; Fakruddin et al. 2015; Sreekanth et al., 2011 and Klich, 2002).

Rapid Aflatoxin determination

In this study aflatoxigenic fungus was detected by Agra Strip total afltoxin test 20ppb cut-off (Mohammad et al., 2019). The Agrastrip total Aflatoxin test is a one-step lateral flow immune-chromatographic assay that determines a qualitative level for the presence of total aflatoxin

in grains, cereals, feeds and other commodities. It detects the presence of aflatoxin at 5 ppb or higher in grain samples by utilizing highly specific reactions between antibodies and aflatoxin in grain samples (Delmulle et al., 2005; Xiulan et al., 2005; Stubblefield et al., 1991). Antibody-particle complex is dissolved in assay diluent and mixed with sample extract. The mixed content is then wicked onto a membrane, which contains a testzone and a control zone. The test zone captures free antibody-particle complex, allowing color particles to concentrate and form a visible line. A positive sample with aflatoxin above the cutoff level will result in no visual line in the test zone. Alternatively, a negative sample with aflatoxin below the cutoff level will form a visible line in the test zone. The line will always be visible in the control zone regardless of the presence of aflatoxin.

Sample preparation / extraction

A representative samples was grinded using a Romer Series II® Mill so that 75% will pass through a 20-mesh screen, then thoroughly mix the subsample portion.10 g of ground sample were taken into a clean jar with 20 mL of 50% ethanol extraction solution (i.e. 50/50 (v/v) ethanol/water) and the jar was sealed and vortexed for 1 minute. The top layer of extract through which sample was filtered by Whatman filter was collected.

Test procedure

All reagents and kit components must be at room temperature 18-30°C (64-86°F) before use. Using a single channel pipette, 50µL of assay diluent to each microwell was added. The coating conjugate was dissolved in the microwell by pipetting the content up and down 5 times. 50µL of sample extracts was added to each microwell, mixing the content in each well by pipetting it up and down 3 times. One test strip was put into one well. The test strip was allowed to develop color for 5 minutes. Then the result was interpreted according to the manufacturer.

RESULTS AND DISCUSSION

For the present study a total of 100 feed samples were collected. Among them 50 are commercially prepared and 50 are self compounded poultry feed. The feed samples were collected from different local farms of Dinajpur district. A series of test were conducted for detection of aflatoxigenic fungus and aflatoxin from poultry feed samples.

In culture media, greenish colonies were found on Sabroud dextrose agar (Figure 1), yellow green with white to cream mycelia and yellow green edges and also in some plates greenish colony were foundon potato dextrose agar (Figure 2), a bright orange colour on the reverse side of the plates on *Aspergillus* differential agar medium which indicated positive result (Figure 3), characteristics conidia were found under microscopic examination by lactophenol cotton blue stain (LPCB) method (Figure 4).

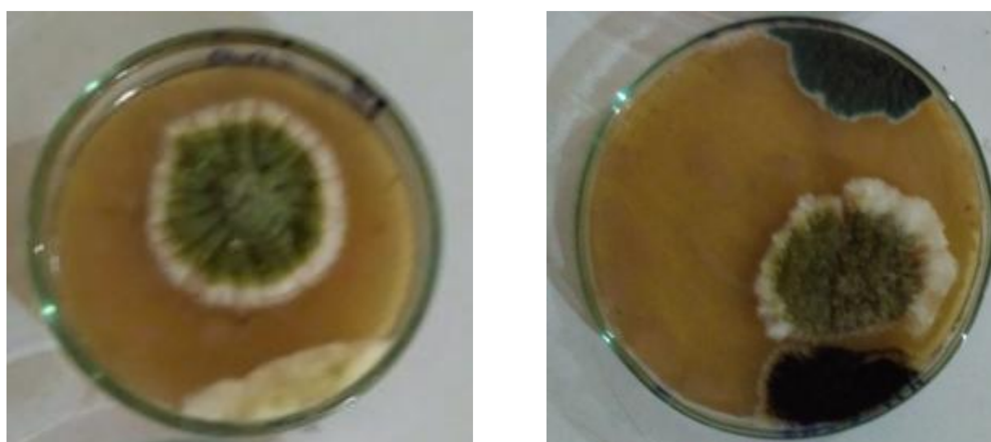

Figure 1: Greenish white colony of Aspergillus *spp* in Sabroud dextrose agar (SDA) Media

Figure 2: *Aspergillus* spp in PDA the colonies were yellow green with white to cream mycelia and yellow green edges and also in some plates greenish colony were found

Table 1: Total prevalence of *Aspergillus spp* and aflatoxigenic fungus in poultry feed

Sample No.	Aspergillus *spp* (Positive case)	Prevalence (%)	Aflatoxigenic fungus (positive case)	Prevalence (%)
1-10	6	60	6	60
11-20	4	40	3	30
21-30	8	80	7	70
31-40	4	40	4	40
41-50	6	60	4	40
51-60	6	60	5	50
61-70	6	60	6	60
71-80	4	40	4	040
81-90	4	40	3	30
91-100	6	60	6	60
Total=100	**54**	**54%**	**48**	**48%**

Table 2: Prevalence of *Aspergillus* spp in self compounded feed on the basis of farm

Farm name	Number of sample examined	Positive number	Prevalence (%)	Chi-square	P-Value
Farm 1	10	6	60		
Farm 2	10	4	40		
Farm3	10	8	80	4.545	.337
Farm 4	10	4	40		
Farm 5	10	6	60		
Total	**50**	**28**	**56**		

Table 3: Prevalence of *Aspergillus* spp according to category of farm in commercially prepared poultry feed

Farm name	Number of sample examined	Positive number	Prevalence (%)	Chi-square	P value
Farm 1	10	6	60	1.923	0.750
Farm 2	10	6	60		
Farm 3	10	4	40		
Farm 4	10	4	40		
Farm 5	10	6	60		
Total	**50**	**26**	**52**		

Table 4: Prevalence of aflatoxigenic fungus in self compounded poultry feed on the basis of farm

Farm name	Number of sample examined	Positive number	Prevalence (%)	Chi- square	P value
Farm 1	10	6	6o	4.327	0.364
Farm 2	10	3	30		
Farm 3	10	7	70		
Farm 4	10	4	40		
Farm 5	10	4	40		
Total	50	24	48		

Table 5: Prevalence of aflatoxigenic fungus in commercially prepared poultry feed on the basis of farm

Farm name	Number of sample examined	Positive number	Prevalence (%)	Chi- square	P value
Farm 1	10	5	50	2.724	0.605
Farm 2	10	6	60		
Farm 3	10	4	40		
Farm 4	10	3	30		
Farm 5	10	6	60		
Total	50	24	48		

Table 6: Agra Strip Total Aflatoxin test 20 ppb cut-off in poultry feed

Number of samples	Aflatoxin level	Line	Remark	Result
5	Non detectable	2 lines	negative	valid
1	15ppb	2 lines	negative	valid
48	≥20ppb	1 line	positive	valid
Total=54	-	-	-	-

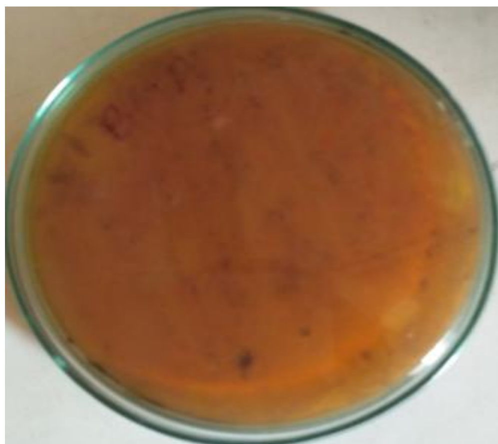

Figure 3: *Aspergillus* spp in *Aspergillus* Differential Agar Base (A bright orange colour on the reverse side of the plates of *Aspergillus* Differential Agar Base indicated a positive result)

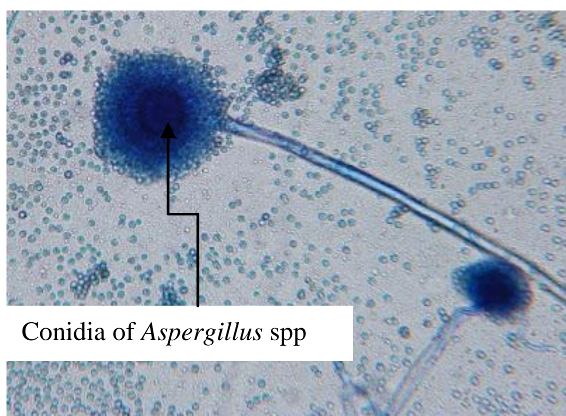

Figure 4: Microscopic Observation of *Aspergillus* spp by Lactophenol Cotton Blue Staining

Among the 100 feed samples *Aspergillus* spp was found on 54 feed samples with 54% prevalence. But Aflatoxigenic *Aspergillus* spp was found on 48 samples with 48% prevalence (Table 1). In this study the prevalence of *Aspergillus* spp was 56% in self-compounded poultry feed and 52% prevalence in commercially prepared poultry feed which was more or less similar to the findings of RM Aliyu et al. 2016.

Prevalence of *Aspergillus spp* in self-compounded poultry feed on the basis of farm had no significant (p>0.05) effect. Farm 3 (80%) had higher prevalence than farm 2 and farm 4 (40%) (Table 2).

Prevalence of *Aspergillus* spp according to farm in commercially prepared feed showed that the prevalence of *Aspergillus* spp was non significantly (p>0.05) highest in farm 1 farm 2 and farm 5 (60%) and lowest in farm 3 and farm 4 (40%) (Table 3)

The study revealed that farm had no significant (p>0.05) effect on the prevalence of aflatoxigenic fungus in self compounded poultry feed. Farm 3 (70%) had higher prevalence and farm 2 (30%) had lower prevalence (Table 4). Whereas the prevalence of aflatoxigenic fungus in commercially prepared feed was non significantly (p>0.05) highest in farm 2 and farm 5(60%) but lowest in farm 4(30%) (Table-5).

For aflatoxin the action levels is 20 parts per billion (ppb) for grain and feed products, and 0.5 ppb for milk established by The Food and Drug Administration (FDA).The level of aflatoxin in raw feed materials could vary from 1 ppb to 680 ppb. In this study maximum value of aflatoxin was ≥20ppb (Table-6), which was more or less similar to the value found by Mohammad et al. (2019). But this result was far from the result found by Fareed (2014) where highest contamination value was 165 ppb among 114 raw feed materials. So it was a great concern for human health because aflatoxin is a potent liver toxin known to cause cancer in animals like reproductive disturbances caused by the T-2 toxin in sows (Placinta et al., 1999) also in human consumer (Richard, 2000).

CONCLUSION

Aflatoxin contamination in feed is a serious risk for public health having long-term health effects. Most of the crops used in culinary in Bangladesh have been reported to be contaminated with aflatoxigenic *Aspergillus flavus* and/or aflatoxins. Continuous surveillance should be conducted to detect aflatoxin contaminated crops and contamination level in different poultry feed. Good agricultural management practice should be employed to reduce contamination risk of aflatoxins and aflatoxigenic fungi in poultry feed. As total eradication of toxigenic fungi and their toxin is not possible, some selective approaches can be a help in aflatoxin management, like, resistant varieties development, biological control

of these fungi and aflatoxin, integrated agronomy practices throughout the whole process of grain harvest, shipping, storage, feed manufacturing, and its formulation. This whole study emphasizes the need of proper surveillance and constant monitoring programs for aflatoxin free food and feedstuffs for human and animal.

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