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Prevalence of Aflatoxin in Maize Grains of Madhubani District - North Bihar

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A B S T R A C T

Mycotoxin contamination is a serious problem of cereal crops in tropical regions like India. Aflatoxin (AF) is one of the most serious mycotoxin contaminants as aflatoxin B_1 is classified as group I human carcinogen. It has mutagenic, tetratogenic and carcinogenic effects. Maize samples from three villages near kosi river, of Madhubani district were collected and examined for associated mycoflora and aflatoxin producing potential of Aspergillus *flavus.* It was found that *Aspergillus flavus* was of dominant occurrence followed by *Aspergillus niger* and species of *Fusarium, Penicillium and Rhizopus.* All fifteen samples, five of each village viz Ghoghardiha, Madhepur and Hatni were found contaminated with aflatoxin, samples collected from Madhepur and Hatni had higher value of AFB_1 contamination (1-250 µg/kg) than of Ghoghardiha village (1-80 µg/kg). 41% of the total *Aspergillus flavus* isolates were toxigenic, producing aflatoxin B_1, B_2 and G_1. The high level of aflatoxin is probably due to favorable environmental conditions, backward agricultural practices, poor storage conditions of grains and due to yearly flood problems in this area.

***Key words*:** Maize seeds, Aflatoxin, Mycoflora, Madhubani district

Maize (*Zea mays* L) is one of the most important cereal crops from the point of agriculture and growing demand of food, as it has wide climatic tolerance, high genetic yield potential and rich nutrient content. It is boosted with antioxidants that help protect consumers from various degenerative diseases [1]. It contributes to 36% global food grain production and is a dominant cereal crop for both livestock feed and human consumption. However agronomic practices and climatic conditions favorable to mould growth severely affect the quality of maize seeds. Main toxigenic fungi associated with maize grains in tropical regions are species of *Aspergillus*, *Fusarium* and *Penicillium* [2-4]. Many mycotoxins are produced by these fungi of which aflatoxin is secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and is group I human carcinogen (ICRA 2002). *Aspergillus* species colonization and aflatoxin contamination is prevalent in tropical regions like India, as growth is favored by high temperature and humidity.

As Bihar is a poor state maize is a staple food and feed of this state. In Bihar, farmers mainly choose rabi (winter) season for maize cultivation because kharif and autumn crop suffer water logging and flood respectively. Madhubani district fall under north-west zone of Bihar and is a flood prone region. Maize is harvested at 20-30% moisture content and lack of advance drying and storage facilities, due to socio-economic backwardness leads to further mould and toxin production [5]. Study which can put light on quality of maize produced in this region is lacking, thus an attempt is made to enumerate the mycoflora associated and aflatoxin produced by isolates of *Aspergillus flavus* in maize samples collected from farmers of Ghoghardiha, Hatni and Madhepur village of Madhubani district.

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MATERIALS AND METHODS

Sample collection

Fifteen samples, of eight month old (from main harvest season) maize seeds were collected from Ghoghardiha, Hatni and Madhepur based farmers and kept in sterile bags for further use. Moisture content of each sample was recorded by help of OSAW moisture meter.

Isolation and identification of Mycoflora

100 kernels of all three-sampling site were surface sterilized in 2% NaOCl and plated on moist blotting paper in sterile petri dishes (ISTA 1966). Enough space was kept between each kernel for proper growth of fungi and avoid cross contamination. Plates were incubated at room temperature for one week followed by macro and microscopic identification [6-8]. Fungal colonies were maintained on PDA media for further use and identification.

Aflatoxin analysis of toxigenic isolates: *Aspergillus flavus* isolates were allowed to grow on SMKY liquid media [9] for 7-8 days at 30±2°C and aflatoxin extracted by the process of Thomas *et al.* [10]. Then it was extracted with chloroform, dried on water bath and concentrated extracts were kept in screw tight bottles for qualitative and quantitative analysis.

Qualitative analysis of aflatoxin was done using thin layer chromatography (TLC), using toluene-isoamyl alcohol – methanol (90:32:2 v/v) solvent system [11]. For chemical confirmation trifluoroacetic acid [12] or 25% sulphuric acid spray was used.

Quantitative estimation of aflatoxin was done spectrophotometrically [13].

Mycotoxin analysis of maize samples: 100gm of maize sample was ground to fine powder and aflatoxin extraction was done by the process of Thomas *et al.* [10]. For qualitative and quantitative analysis same process was followed as that mentioned above for *Aspergillus flavus* isolates.

RESULTS AND DISCUSSION

Each maize seed plated was carefully examined for fungal growth and associated mycoflora were noted which include *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Fusarium sp.*, *Penicillium sp*, and *Rhizopus stolonifer* (Table 1).

The (Table 2) represents the data of toxigenic isolates of *Aspergillus flavus* obtained from SMKY liquid medium, type of aflatoxin produced and range of aflatoxin B_1 produced by these isolates. Total 197 isolates of *Aspergillus flavus* was screened for aflatoxin producing potential and 81 isolates were found positive. Out of 81 isolates, 57 produce AFB_1, 18 produces both AFB_1 and AFB_2 and only 6 isolates produce AFB_1, AFB_2, and AFG_1 however no isolate was found to be positive for AFG_2. In the isolates of sample collected from Ghoghardiha amount of aflatoxin B_1 ranged between 0.2-5ppm whereas samples of Madhepur and Hatni village had higher amount of aflatoxin B_1 contamination, in the range of 0.2-10ppm. A very few isolates (13%) produced AFB_1 above 5ppm and remaining isolates were low producers of AFB_1.

Maize grains were analyzed for the presence of different types of aflatoxin and the quantity of aflatoxin B_1 present, (Table 3) shows that range of AFB_1 in samples collected from Ghoghardiha village is comparatively low (1-80µg/kg) from that collected from Madhepur and Hatni villages (1-250µg/kg). All samples were positive for AFB_1 and AFB_2 and AFG_1 was additionally present in samples of Madhepur and Hatni. Moisture content of samples collected from Ghoghardiha, Madhepur and Hatni villages are 8.8, 10.2, and 9.1 respectively.

Table 1 Mycoflora associated with maize seeds

Mycoflora	Ghoghardiha (N=100)		Madhepur (N=100)		Hatni (N=100)	
	Number of seeds infested	Moisture content	Number of Seeds infested	Moisture content	Number of seeds infested	Moisture content
Aspergillus flavus	60		73		64	
Aspergillus niger	5		8		2	
Fusarium moniliforme	1	8.8	0	10.2	2	9.1
Fusarium sp	4		5		1	
Penicillium sp	0		4		3	
Rhizopus stolinifer	18		14		23	

Table 2 Aflatoxin producing potential of toxigenic isolates of *Aspergillus flavus*

Place of collection	Total *Aspergillus flavus* isolates	Number of isolates producing aflatoxin	% positive isolates of *Aspergillus flavus*	Type of Aflatoxin			Range of AFB1 (ppm)
				B_1	B_1B_2	$B_1B_2G_1$	
Ghoghardiha	60	26	43	18	8	-	0.2-5
Madhepur	73	32	44	25	4	3	0.2-10
Hatni	64	23	36	14	6	3	0.2-10
Total	197	81	41	57	18	06	

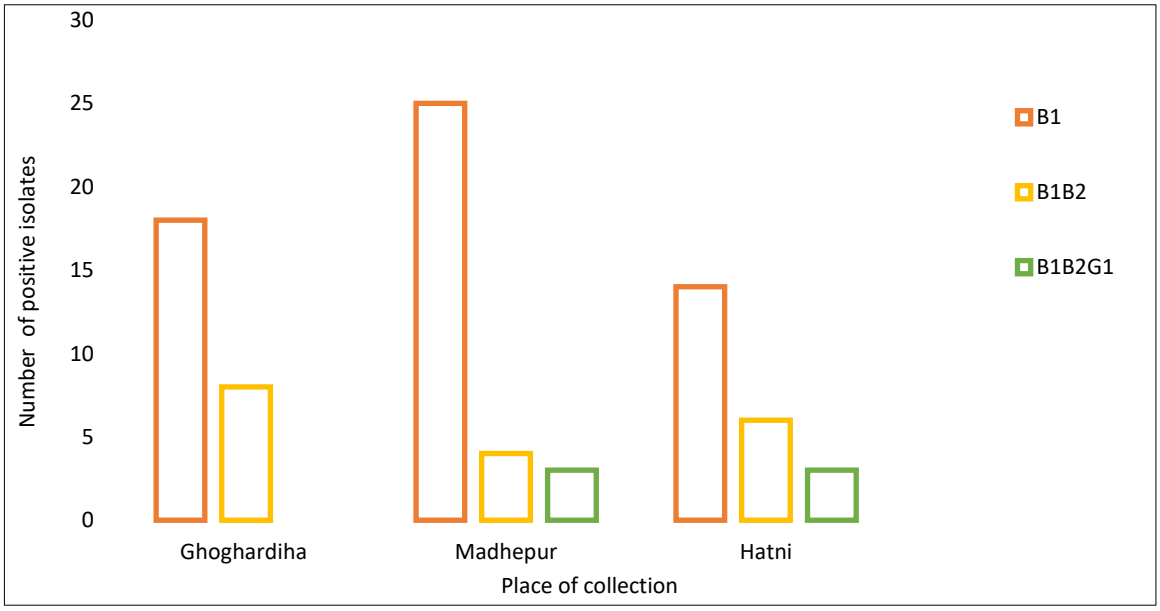

Fig 1 Extent of different aflatoxin produced by toxigenic isolates of *Aspergillus flavus*

Table 3 Aflatoxin contamination in maize grains

Place of collection	Moisture content	Sample positive for Aflatoxin			Range of AFB_1 (µg/kg)
		B_1	B_2	G_1	
Ghoghardiha	8.8	+	+	-	1-80
Madhepur	10.2	+	+	+	1-250
Hatni	9.1	+	+	+	1-250

This study clearly indicates the association of mycotoxin producing fungi in maize seeds, viz., *Aspergillus*, *Penicillium* and *Fusarium*, the dominance of *Aspergillus* concurrence with earlier studies performed in the state of Bihar [14-15]. High aflatoxin level is probably due to favorable environmental conditions, poor storage, lack of use of natural and chemical control methods due to absence of knowledge and poverty.

Most of the maize crop produced by the farmers of this region is used either as food or feed requirement of their own or sold in local markets and dearth of knowledge and proper checks is somehow affecting the health of these people without being noticed, can lead to aflatoxicosis outbreaks, thus there is a need for better management by using techniques like IPM (Integrated Pest Management) which can eliminate or at least bring down the level of *Aspergillus flavus* and consequently aflatoxin contamination in maize grains produced in Madhubani district of Bihar [16].

CONCLUSION

All samples tested in this study were found dangerously contaminated with aflatoxin, but no awareness among farmers and common people about the side effects of consumption of such contaminated food is opening ways for contamination of entire food chain with aflatoxin. This brings the need for intense research and application of different control methods of *Aspergillus* contamination in modern agriculture.

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