Original Research Article

Surveillance of Mycotoxigenic Fungi and Production of Aflatoxin by *A. flavus* in Contaminated Maize Seeds- Bihar.

Abstract

Aflatoxin- producing fungi like *Aspergillus flavus* contaminate maize crops in the agricultural field at harvest, post- harvest and during storage making them one of the most widespread and dangerous mycotoxins. It has been directly correlated to the adverse ~~the~~ health effects, such as liver cancer in many animal species as well as plant system.

Maize samples collected from seven districts of Bihar viz, Begusari, East champaran, Khagaria, Bhagalpur, Saharsa, Madhepura and Samastipur. Collected sample were examined for associated mycoflora and aflatoxin producing prospective of *Aspergillus flavus*. It was determined that *A. flavus* was of presiding occurrence accompanied by *Penicillium spp., Fusarium, A. nigar, Rhizopus, Trichoderma, Mucor* and *A. ochraceus*. 119 strains of the total *A. flavus*isolates were toxigenic, producing aflatoxin B_1, B_2 and G_1. Highest Aflatoxin is all probability due to complementary environmental conditions, undeveloped agricultural practices, poor storage circumstances of grains and because of yearly flood problems in this region.

Key words: Maize seeds, Aflatoxin, Mycoflora, ~~Seven districts of~~ Bihar.

INTRODUCTION

Mycotoxin (Greek word Mykes = Fungus and Toxin = Toxin) is toxic secondary metabolite produced by organism of Kingdom Fungi (Richard JL, 2007; Cimen*et. al.*, 2022) and is capable of causing disease and death in humans and animals (Bennett, J. W and Klick, M. 2003). They are stable, invisible and toxic chemical compounds, found to be common in the farm environment surviving in several places as well as on many different types of feed stuffs.

The growing moulds and production of mycotoxin are most encouraged by environmental factors like temperature (cool / hot) and moisture (wet / dry). However, mycotoxins may also be produced by moulds when other stress conditions occur to the host plant or the mould.

Aflatoxins are a type of mycotoxin produced by *Aspergillus* spp.such as *A. flavus*and *A. parasiticus*(Martins *et. al.*, 2001). The umbrella term aflatoxin refers to four different compounds which are B_1, B_2, G_1 and G_2 (Yin YN *et. al.*, 2008) where Aflatoxin B_1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as Liver cancer in many animal species (Martins *et. al.*, 2001).Aflatoxin is largely associated with commodities

produced in the tropics and subtropics region in cotton, wheat, millet, spices, rice, sorghum, peanuts, sunflower, pistachios and maize crops (Martins *et. al.,* 2001; Yin YN *et. al.,* 2008).

Maize is widely cultivated throughout the world and a greater weight of maize is produced each year than any other grain (International grains council, 2013). In 2021, total world production was 1.2 billion tonnes. Maize is the most widely grown grain crop throughout the America. Out of 38 administrative districts of Bihar only seven districts, viz. Begusarai, East champaran, Khagaria, Bhagalpur, Saharsa, Madhepura and Samastipur constitute about half of the total maize ~~acceage~~ acreage. These districts are historically flood- prone areas during rainy season and fall north of the Gangas, having several seasonal river tributaries.

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These seven districts of Bihar have recorded in April 2021, the highest maize productivity in the world. Maize cultivation provides livelihood to approx. 1.3 million farmers of Bihar state. Maize has become a staple food in many parts of the world with the total production surpassing that of wheat or rice.In addition to being consumed directly by humans (often in the form of masal).Maize is used for corn ethanol, animal feed and other products, such as corn starch and corn syrup (Foley, J. 2019). It is also used in making ethanol and other biofuels.

Therefore, an attempt has been made to surveillance of mycotoxigenic fungi and production of aflatoxin by *A. flavus* in contaminated maize seeds of major growing districts of Bihar.

Occurrence of mycotoxin in maize crop

Aflatoxin – producing fungi like *A. flavus*contaminate maize crops in the agricultural field at harvest and during storage making them one of the most widespread and dangerous mycotoxins. In Kenya (2004), about 125 people died and nearly approx. 200 others required medical treatment after eating aflatoxin contaminated maize (Lewis L. *et. al.,* 2005). The death was associated with home-grown maize that had not been treated with fungicides or properly dried before storage. At that time, due to food shortages, farmers may have been harvesting maize earlier than normal to prevent thefts from their agricultural field, so that the grain had not fully matured and was more susceptible to infection with *A. flavus.*

MATERALS AND METHODS

Sample collection

Maize seed samples were collected from farmers field in seven districts viz.Begusarai, East champaran, Khagaria, Bhagalpur, Saharsa, Madhepura and Samastipurof Bihar. Maize seed samples kept in sterile polythene bags for further experiments. Moisture content of each sample was recorded with the help of OSAW moisture meter and ~~PH~~ pHreading were taken by using digital ~~PH~~ pHmeter (IJBAF, 2013).

Isolation and identification of Mycoflora

100 kernels of all seven- sampling site (districts)was surface sterilized in 2% Naocl and plated on moist blotting paper in sterile petri dishes (ISTA, 1966). Plates were incubated at room temperature for 7 days followed by macro and microscopic identification (Mc clenny, N. 2005; Adame- Garcia, J. *et. al.,* 2015). 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 (Diener and Davis, 1966) for one week at 30 ± 2˚c and aflatoxin extracted (Thomas *et. al.*, 1975). It was extracted with chloroform, dried on water bath and concentrated extracts were kept in screw tight bottle for qualitative and quantitative analysis.

Qualitative and Quantitative analysis

Qualitative analysis of aflatoxin was done using TLC (Thin layer chromatography), toluene- isoamyl alcohol- methanol (90: 32: 2 v/v) solvent system (Reddy *et. al.*, 1970) and for chemical conformation trifluoroacetic acid (Stack and Pohland, 1975) or 25% sulphuric acid spray was used.

Quantitative estimation of aflatoxin was done by spectrophotometrically (Nabney and Nesbitt, 1965).

RESULTS

Table -1 and fig. A, indicates the association of mycoflora in seven districts of Bihar in flood – prone area during rainy season (Agroclimaic zone in Fig- B)and observed during the study, moisture content and PH of the sample. *A. flavus*, *Penicillium* spp., *Fusarium*, *A. nigar*and *Rhizopus* were present in all the seven districts in large amount whereas *Trichoderma*, *Mucor* and *A. ochraceus*present in rare amount of all districts but absent in Khagaria and Saharsa districts, respectively (Fig- C). PH ranged between 5.8 to 6.7 and moisture content were recorded 10.8, 10.5, 10.0, 9.6, 10.1, 10.4 and 10.7 for Begusarai, Eastchamparan, Khagaria, Bhagalpur, Saharsa, Madhepura and Samastipur, respectively.

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Table-2 represent that *A. flavus* sample of 7 districts having 385 isolates of *A. flavus*. 119 strains were found to be toxigenic in which86 positives to AFT- B_1, 24 positives to AFT-B_1B_2 and only 9 positives for AFT-$B_1B_2G_1$. The amount of AFT-B_1 was, however very low at 0.2- 1.3, 0.0- 1.0 and 0.0- 1.1 µg/ml, respectively.Whenever, the maximum aflatoxin was recorded from Begusarai

samples at 0.2- 1.8 µg/ml followed by East champaran, Khagaria, Saharsa, Bhagalpur, Samastipur and Madhepuradistrict, respectively.

Maize samples collected from seven districts in flood- prone area during rainy season were highly contaminated with *A. flavus* and other genera of fungi like, *Penicillium, Fusarium, A. nigar, Rhizopus, Trichoderma, Mucor* and *A. ochraceus*. Mycotoxin producing fungi like *Aspergillus, Fusarium* spp. were of predominant occurrence (Fig. Dand E). The moisture contents were also observed in all the samples which influence the aflatoxin production.

DISCUSSION

Recently, about 125 people died and nearly approx. 200 others required medical treatment after eating aflatoxin contaminated maize (Lewis L. *et. al*., 2005). In Kenya (2004), the death was associated with homegrown maize that had not been treated with fungicides or properly dried before storage. At that time, due to food shortages, farmers may have been harvesting maize earlier than normal to prevent thefts from their agricultural field, so that the grain had not fully matured and was more susceptible to infection with *A. flavus*and aflatoxin producing potentiality of toxigenic strains of *A. flavus* were higher, due to moisture content in rainy season in flooded area as well as poor storage conditions that provide an opportunity for fungal growth like Aflatoxin to easily ~~envade~~ invadethe maize seeds.

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Bihar has experienced serious aflatoxicosis outbreaks associated with maize which has claimed lives as well as maize yield losses. Our findings from the various sites (districts) revealed that, potentially mycotoxigenic fungal isolates were found on maize samples. Maize samples from each district were more infested by a specific fungal genus. Maize grains from Begusarai were heavily contaminated by *Aspergillus* spp. while those collected from Khagaria and Saharsa were not contaminated with *Tricoderma*and *Mucor*.

CONCLUSION

Maize samples from the seven districts tested were infested by different mycotoxigenic fungi. The existence of mould on the maize samples shows the possibility of occurrence of more than one mycotoxin but dominant by Aflatoxin B_1. ~~This may be~~ The maize has the potential to enhance the income of 1.3 million maize grower in Bihar State, thereby significantly reducing poverty ~~in us~~ of the poorest ~~states~~ stateIn India.

So, it was important to determine the distribution and incidence of fungi that exist in maize from different districts.

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Comment [g5]: Arrange alphabetically

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Table -1:Mycoflora associated with Maize Seeds collected from seven Districts of Bihar

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Sl. No.	Fungus	Maize Seed Samples from Seven District																					
		Begusari (N =100)			EastChamparan (N =100)			Khagaria (N =100)			Bhagalpur (N =100)			Saharsa (N =100)			Madhepura (N =100)			Samstipur (N =100)		
		No. of seed infested	Moisture content	PH	No. of seed infested	Moisture content	PH	No. of seed infested	Moisture content	PH	No. of seed infested	Moisture content	PH	No. of seed infested	Moisture content	PH	No. of seed infested	Moisture content	PH	No. of seed infested	Moisture content	PH
1	*A. Flavus*	75	10.8	6.7	66	10.5	6.3	63	10.0	6.0	57	9.6	5.8	46	10.1	5.9	41	10.4	6.2	37	10.7	6.5
2	*Penicillum Spp.*	68			60			57			49			42			38			33		
3	*Fusarium Spp.*	62			58			43			34			30			28			25		
4	*A. niger*	74			65			62			56			45			40			36		
5	*Rhizopus Spp.*	35			32			24			21			19			16			13		
6	*Trichoderma*	9			5			0			3			6			9			4		
7	*Mucor*	4			9			1			7			0			1			5		
8	*A. ochraceus*	5			8			0			4			7			2			1		

Table- 2: *Aspergillus flavus* isolates from Maize Seed samples

Sl. No.	Districts of Bihar	No. of *A. flavus* strains isolates	No. of toxigenic isolates of *A. flavus*	Positive isolates			Range of aflatoxin B_1 concentration µg/ml (ppm)
				B_1	B_1 B_2	B_1 B_2 G_1	
1	**Begusari**	75	25	15	7	3	0.2- 1.8
2	**East Champaran**	66	19	13	5	1	0.1- 1.6
3	**Khagaria**	63	18	12	4	2	0.2- 1.4
4	**Bhagalpur**	57	13	11	1	1	0.2- 1.3
5	**Saharsa**	46	17	14	2	1	0.0- 1.2
6	**Madhepura**	41	12	10	2	0	0.0- 1.0
7	**Samstipur**	37	15	11	3	1	0.0- 1.1
	Total	**385**	**119**	**86**	**24**	**9**	-

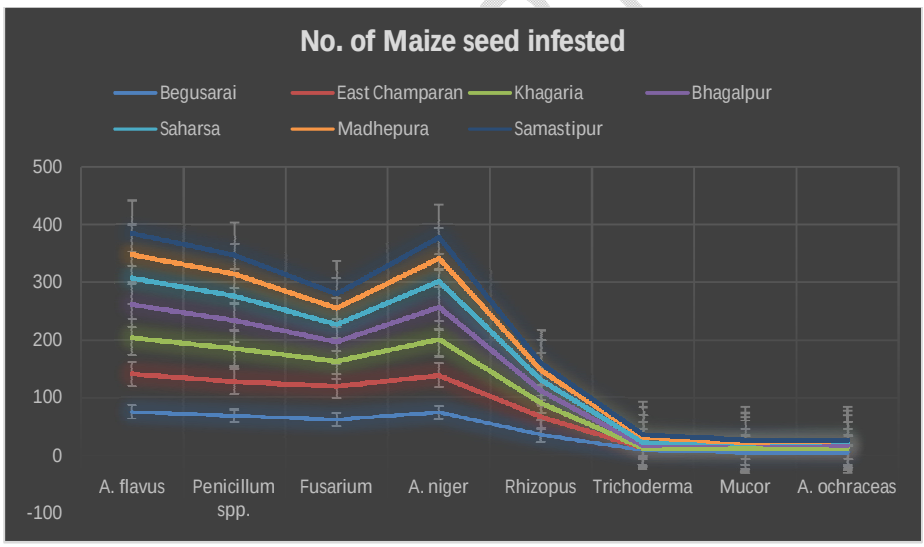

Fig- A: Line showing No. of Maize Seed infested in Seven Districts of Bihar

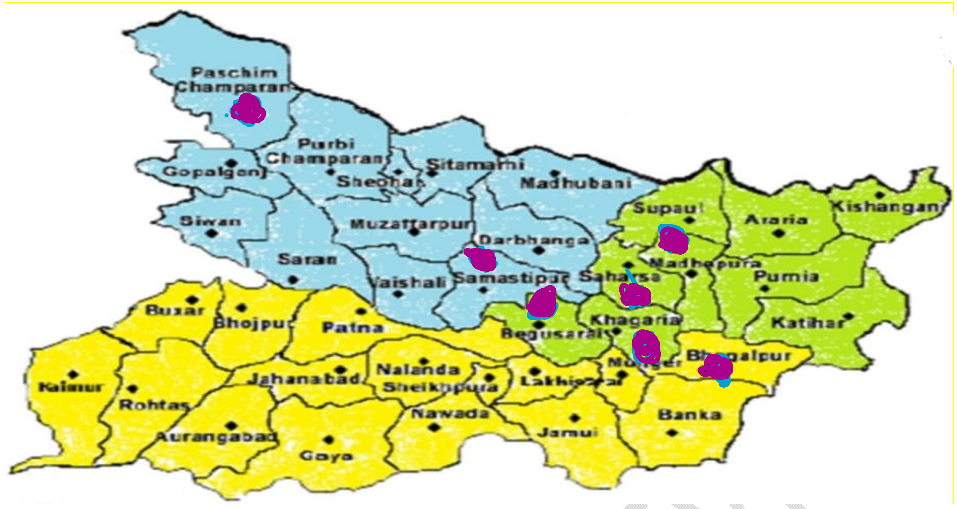

Fig B: **Pink dot indicates Agroclimatic Zone of Seven Districts of Bihar**

Fig- C: Association of Mycoflora in PDA media of Seven Districts of Bihar

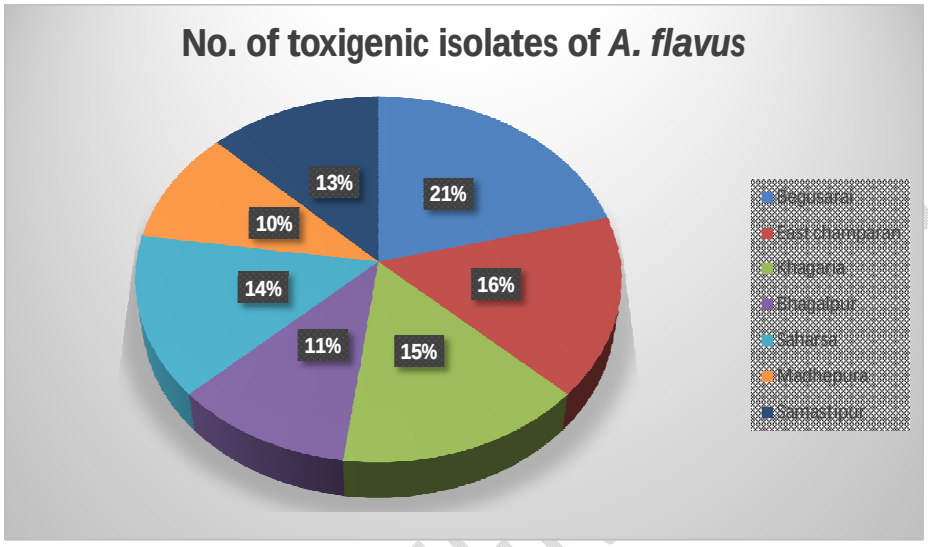

Fig- D: Pie chart showing No. of toxigenic isolates of *A. flavus* (%) in Seven DistriBihar

Fig- E: **Association of Mycoflora in maize comb during flood situation**