Mycobiota and aflatoxin production in maize seeds (*Zea mays L.)* from Darbhanga District – North Bihar.

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Abstract:
Maize (*Zea mays L.)* is an important cereal crops in this state, Bihar. It becomes contaminated with different harmfull mycotoxin. Mycobiota and aflatoxin contamination were determined in stored maize samples collected from three villages of Darbhanga District– N. Bihar to know seed quality . A total of 207 fungal isolates of *A. flavus* were observed in all the samples in which 56 isolates of *A. flavus* were positive for AFB_1 production .The range of AFB_1 concentrations were observed from 0.2-5, 0.2-8 and 0.2-10 µg/ml in Sakri , jale and Kusheshwar Asthan , respectively .The maximum toxigenic strains of *A. flavus* were recorded in Kusheshwar Asthan followed by Jale and sakri . The moisture contents was also observed in all the samples ,which influence the aflatoxin production.

Key words: Mycobiota, Mycotoxin, Maize seeds, Moisture content and pH values.

INTRODUCTION:

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide after wheat and rice (Golob *et al.*, 2004). In India, 15 million farmers are engaged in maize cultivation, especially in Karnataka, M.P, Maharastra and Bihar state (Sinha *et. al.*, 2018).The production of maize in Bihar approximately 7.21 lakh / hectares. It has been established that the moisture contents (about 18-20%) and temperature (25-30°C) are two environmental factors that influence, the growth of various fungi in maize (Sinha, 1990; Egal *et. al.*, 2005; Alborch *et. al.*,2011).The major fungal genera encountered on maize seeds are *Fusarium* , *Aspergillus* and *Penicillium* during rainy season as well as flooding situation in tropical regions (Sinha *et. al.*,1987 ;Khosravi *et. al.*,2007). It means monsoon climates condition has proved very favorable for the growth of toxigenic and non-toxigenic fungi in harvesting, storage and transportation of maize seeds.

Aflatoxin produced by *A. flavus* Link Ex Fries is the most toxic in all the mycotoxins studied (Anonymous, 1979). Aflatoxin has been reported as natural contaminants in consumable items (Hessetline 1976; Bilgrami and Sinha 1984) constiquency global threat to human beings and livestock.

Moreover, very few research works have been done in Darbhanga -North Bihar to evaluate the health status of maize seeds from formal and informal seed production sectors. In view of the above mention facts, the present research work has been undertaken to record the associated mycobiota as well as production of aflatoxin by A. *flavus in* maize seeds.

MATERIAL AND METHODS:

SAMPLE COLLECTION:

Maize samples were collected from three different places like : Sakri , Jale , Kusheshwar Asthan village in Darbhanga district from storage during monsoon seasons .The samples were labelled, packed in sterile polythene bags and transferred to the laboratory, for further research work.

ISOLATION OF FUNGI:

Seed borne mycoflora of maize were tested by using standard Blotter Paper test ISTA (1999). 100 seeds of all three samples were surface sterilized with 0.2% NaOCl solution for 10 minutes and plated on moist Blotting Paper in sterile petri dishes in triplicate. The plates were incubated at room temperature (28±2°C) for 7 days and then developing colonies were examined for fungal growth under a stereomicroscope.

DETERMINATION OF MOISTURE CONTENT AND pH OF SEED SAMPLES:

The moisture contents were determined by standard analytical methods (OSAW moisture meter; AOAC-1980). The pH reading were taken by using digital pH meter. (Digital thermo pH meter mob-B-E105). (IJBAF, 2013).

EXTRACTION OF AFLATOXIN:

Aflatoxin producing potentials of *A. flavus* group of fungi (Raper and Fennell; 1965) were tested in SMKY liquid medium (Diener and Davis, 1966).
Aflatoxin were finally extracted with chloroform and the extracts were used for further qualitative and quantitative analysis.

QUALITATIVE AND QUANTITATIVE ANALYSIS OF AFLATOXIN:
Qualitative analysis of aflatoxin was done on a Thin Layer Chromatography plate (TLC) by using toluene-isoamyl alcohol-methanol (90:30:2.v/v) system (Reddy *et. al.*,1970). The presence of aflatoxin B1 in the contaminated samples was chemically confirmed by using trifluoroacetic acid (Stack and Pohland 1975).
The quantitative estimation of AFB1 was done by spectrophotometrically (Nabney and Nesbitt, 1965).

RESULT AND DISCUSSION:
Table 1 indicates the associated mycoflora / Mycobiota observed during the study, moisture content and pH of the sample .*A. flavus* , *A. niger* and species of *Penicillum*, *Fusarium* and *Rhizopus* were present in all the 3 samples where as *A. ochraceus* and *Alterneria* were absent in Jale and Kusheshwar Asthan village, respectively. pH ranged between 6 to 6.6 and moisture content recorded was 10.1, 9.8, 10.9 for Sakri, Jale , Kusheshwar Asthan village , respectively.

Table :1 –**Mycobiota associated with maize seeds collected from Darbhanga District**

Fungus	Sakri (N=100)			Jale (N=100)			Kusheshwar Asthan (N=100)		
		Moisture Content	pH		Moisture Content	pH		Moisture Content	pH
1) A. flavus	64	10.1	6.6	68	9.8	6.4	75	10.9	6
2) A. nigar	52			44			28		
3) *Alternaria spp.*	6			0			5		
4) *Penicillium spp.*	18			11			9		
5) *Rhizopus arrhizus*	5			8			6		
6) A. ochraceas	4			9			0		
7) *Fusarium spp.*	33			27			6		

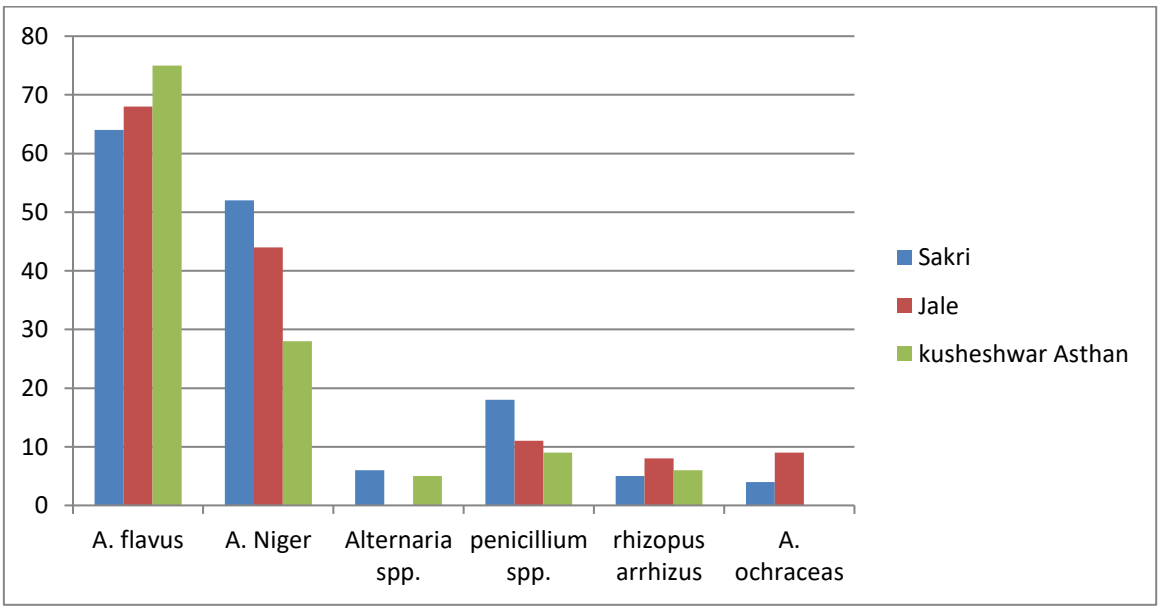

FIG 1 : **Density of Mycobiota with maize seeds**

Table -2 represent that in 3- samples of the maize seed having 207 isolates of . *A. flavus*, 71 were found to be toxigenic and 56 positive to AFB_1, 13 positive to AFB_1B_2 and only 2 positive for $AFB_1B_2G_1$. The amount of AFB_1 was, however very low, only 0.2-10µg/ml. The maximum aflatoxin was recorded from Kusheshwar Asthan samples followed by Jale and Sakri villages , respectively where as AFG_1 is absent in Kusheshwar Asthan samples.

Table -2 : *Aspergillus flavus* isolates from maize seed samples

Fungus	No. of *A. flavus* Strains isolates	No. of toxigenic isolates of *A. flavus*	Positive isolates			Range of aflatoxin B1 Concentration µg/ml
			B1	B1B2	B1B2G1	
A. Flavus (Sakri)	**64**	**21**	**18**	**2**	**1**	**0.2-5**
A. flavus (Jale)	**68**	**23**	**16**	**6**	**1**	**0.2-8**
A. flavus (Kusheshwar Asthan))	**75**	**27**	**22**	**5**	**_**	**0.2-10**
Total	**207**	**71**	**56**	**13**	**2**	**-**

The maize samples collected from stores during rainy season were highly contaminated with *A.flavus* and other genera of fungi like, *Penicillium*, *Fusarium*. Mycotoxin producing fungi like Aspergillus, *Penicillium and Fusarium* spp. were of predominant occurrence. (Fig-1). The moisture contents was also observed in all the samples which influence the aflatoxin production.

DISCUSSION:

The aflatoxin producing potentiality of toxigenic strains of *A. flavus* were fairly high, probably due to moisture content in rainy season as well as poor storage conditions provide an opportunity for growth of fungi to easily envade the maize seeds.

CONCLUSION:

All samples tested in this study were found dangerously contaminated with aflatoxins .It is an important task to control mycotoxin producing fungi from the stored maize seeds by maintaining proper hygiene and drying seeds to eliminate/prevent/inactivate the *A. flavus* infection and aflatoxin contamination in maize seeds. The above result reveals that mycotoxin contaminated seeds are therefore, not suitable for consumption as well as for sowing purposes, because of crop yield will greatly be hampered due to so many abnormalities in the physiology of crop plants. One should be very cautious in selecting the seeds to be used for crop cultivation.

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REFERENCES:

1. Alborch. L., M. R. Bragulat, M. L. Abarch ,and f. j. Cabanes. 2011. Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillusniger* and *Aspergillus* carbonarious on maize kernels. *International journal of food Microbiology* **147(1):** 53-57.
2. ANONYMOUS, 1979. Mycotoxin ,Genera, WHO publication ; 127.
3. AOAC, 1980. In: official Methods of Analysis of the Association of Analytical Chemists ,13th Ed., AOAC , Washington D.C., 1980, 143-260.
4. Bilgrami, K. S .and Sinha, K. K.1984. Mycotoxin in cereals. Trop. *Plant pathol.*,**11:**335-374.
5. Diener, U.L. and Davis, N.D. 1966. Aflatoxin production by isolates of *Aspergillus flavus. Phytopathology*, **56**, 1390-1393.

6. Egal, S., Hounsa A. , Gong Y. Y., Turner P. C., Wild C. P., Hal A. J., Hell K., and Cardwell K. F.. 2005. Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. *International Journal of Food Microbiology* **104(2):** 215– 224.
7. Golob, Kutukwa P. N., Devereau A. , Bartosik R. E., and Rodriguez J. C . 2004. Chapter two: Maize.*Crop Post- Harvest: Science and Technology,Volume* 2. R. Hodges, and G. Farrell, eds. Ames, Iowa. Blackwell Publishing Ltd.
8. Hesseltine, C.W. 1976, In Proceedings of the Third International Biodegradation Symposium (Ed., sharply J. M. and Kaplan, A.M.) PP 607-623, *Applied Science Publishers*,London.
9. IJBAF 2013. Mycoflora and aflatoxin contamination of some foodstuffs *ORANUSI SU AND OLAREWAJU SA **, 1(1**):9-18.
10. ISTA 1999 International Rules for Seed Testing. *Seed Sci. Tech*. **23**:269.
11. Kosravi A. R., Mansouri M, Bahonar A.R. Shokri H. 2007. *Pakistan journal of Biological Science* .
12. Nabney, j., and Nesbitt B. F. 1965. A spectrophotometric method for determining the aflatoxins Analysis **90**:155-160.
13. Raper, K. B and Fennell, D. I. ,1965, The Genus *Aspergillus* (Baltimore :Williamsand Wilkins), p.686.
14. Reddy, T.V., Viswanathan, L. and Venkita Subramania,T.A. 1970. Thin layer chromatography of aflatoxins. *Analytical Biochemistry*, **38:** 568-571.
15. Sinha S. B. ,Kasana R. K. and Sinha. S. P. 2018. Status of corn cultivation in bihar:opportunities and future Challenges.
16. Sinha, K. K. 1987, Aflatoxin Contamination of maize in flooded areas of Bhagalpur, India.
17. Sinha K. K. 1990 .Incidence of mycotoxins in maize grains in Bihar State ,India. *Food Addit Coontam* **7;** 55-61.
18. Stack, M. E., and Pohland. A. E. .1975. Collaborative study of a method for chemical conformation of identity of aflatoxin. *J. Assoc. Off. Anal. Chem.* **58**:110-113.

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