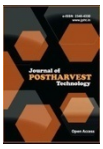

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RESEARCH ARTICLE

Assessment of fungal diversity and impacts of aflatoxin B1 on physiology of maize seeds (*Zea mays* L.)

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ABSTRACT

Maize seeds samples were collected from three locations of Darbhanga viz. Kusheshwar Asthan, Sakri and Jale with respect to mycoflora, toxin production and maize seedling growths. The number of mycoflora *Aspergillus flavus, Fusarium* spp*, A. niger, Penicillium spp.* was recorded from all the locations maize seeds. However, among all the mycoflora, A. *flavus* was found to be dominant. Basically aflatoxin B_1, B_1B_2, $B_1B_2G_2$ were also produced by *A. flavus* in SMKY Liquid media, ranging between 0.2 and 2.7 µg/l. The impact of B_1 on seed germination and seedling growth was also recorded. The maximum inhibition in seed germination were observed at 2000 ppm by variety A (75.26%) and variety B (76.66%) respectively. The maximum inhibition in root and shoot lengths was also noticed in variety A (79.49%, 68%) and Variety B (71.07%, 59.71%) at 2ppm of aflatoxin B_1, respectively over all my observation conclusive that the healthy seeds is the foundation of healthy plant a necessary condition for high yields.

Keywords: Maize seeds, seed-borne, mycoflora, germination, seedling growth.

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INTRODUCTION

Maize *(Zea mays L.)* is the most widely distributed crop of the world, especially in developing countries like India. It is a traditional growing plants in U.P, M.P, Jharkand and Bihar accounting about half of the total production share at National level (Joshi et al., 2005). In Bihar, Maize is cultivated at about 7.21 Lakh hectares with production of 28-20 Lakh tones (DES-GOB-2020-21).

Maize is mostly used by as major sources of feed and industrial row materials at the global levels i.e., Staple food (17%), as feed (61%) and industrial purposes (22%). However, In India , it is also highly used for poultry starch and cattle feed (21%) (Bathla et al., 2019).

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The crop is infected and affected by different microbes like Aspergillus*, Penicillium, fusarium* spp. due to suffer water logging and flood situations which not only impede its grown but also cause loss of high yields. Darbhanga is one of the district fall under north –west zone of Bihar is a flood prone regions. (Diksha et al., 2022). Various pathogenic fungi recorded by producing mycotoxin on maize seeds. (Park et al., 2005) AFB1 is one of the mycotoxin produced by A. flavus were observed during storage system and consequently impacts on nutritive values (Park et al., 2004 ; Koirala et al., 2005; Domijan et al., 2005).

AFB_1 has also been restricted the plant physiology like; inhibition in seed germination, seedling growth as well as other physiological process of plant (Sinha and kumari 1990; Prasad et al., 1996). Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on plant physiology (Khlangwiset et al., 2011).

Only few reports are available on maize varieties (Pioneer and White maize - X08) therefore, an attempt has been made in the present investigations to record the comprehensive studies of fungal diversity and production of Aflatoxin by *A. flavus* on physiology of maize seeds (varieties - Pioneer and White maize - X08) in Darbhanga District, Bihar.

MATERIALS AND METHODS

Sample Collection

Seed sample of different varieties were collected in three random sampling sites (Kusheshwar Asthan, Sakri and Jale) from the field crops of Darbhanga Districts. The samples were transported in sterilized polythene bags for further analyzing of mycoflora and toxin production.

Isolation and Identification of fungi from seeds

100 seeds were used for isolation and identification of seed borne fungi .Seeds were surface sterilized with 2% NaOCl for 1 minute and rinsed with distilled water and plated on blotting paper by the methods of ISTA (1999).

SMKY Liquid Media

Aflatoxin producing potentiality of *Aspergillus flavus* isolates were grown on SMKY liquid media (Diener and Davis 1966) . After 7-8 days at 28±2▫C aflatoxin were extracted by the process of Thomas et. al. (1975).

Seed germination and Seedling growth

The concentration of AFB_1 were used as 2000 ppb , Since the effects of 5 different concentration of AFB_1 (control, 100, 250, 500, 1000, 2000 ppb) on seed germination and seedling growth of maize seeds were studying during Ph.D work (Prasad, 1992). The maximum inhibition in above parameters were recorded at 2000 ppb concentration of Aflatoxin B_1.

100 healthy maize seeds of two varieties were soaked in double distilled water for 24 hrs. The soaked seeds were Petri plates will be kept in the incubated in seed subsequently placed on moist blotting paper in triplicate sets . Then, the germinator at 28± 2▫C at light cycle 12/12 h and data will be recorded after 5 days. On the basis of the germination index (G.I) was calculated by following formula:

$$\text{G. I.} = \frac{\text{Total no. of germination seed}}{\text{Total no. of seed observed}} X\ 100$$

After germination of 7 days the shoot and root lengths were measured with scales. This data was analyzed statistically i.e., t-test for seed germination and F-test for seedling growth, statistical calculations were carried out using the ANOVA test (Dospekhov, 1984).

Table 1: Fungal diversity on maize seeds var. A (Pioneer) and B (White maize -X08) in various places.

Fungal sps.	Maize variety A (Pioneer)				Maize (White maize Variety B X08)			
	Kusheshwar Asthan	Sakri	Jale	Mean (S.E)	Kusheshwar Asthan	Sakri	Jale	Mean (S.E)
A. flavus	77	65	58	66.66±1.2	68	60	53	60±2.47
A. niger	21	30	18	7	19	6	4	
Alternaria spp.	5	0	4		9	7	6	
Fusarium spp.	4	9	5		7	11	0	
Penicillium	8	4	9		0	12	9	
Rhizopus	14	11	4		10	5	0	

Table 2: Production of aflatoxin by *A. flavus* in SMKY liquid media

No. of *A. flavus* isolates sample	Sites	No. of *A flavus* Strain isolates	No. of toxigenic isolates of *A. flavus*	Type of aflatoxin produce (positive)			Range of aflatoxin B1 Concentration (µg/l)
				B_1	B_1B_2	$B_1B_2G_1$	
Variety A (Pioneer)	Kusheshwar Asthan	77	28	12	8	8	0.2-1.8
	Sakri	65	17	8	8	1	0.2-1.6
	Jale	58	14	8	6	-	0.2- 2.7
Variety (White maize X08)	Kusheswar Asthan	68	18	10	6	2	0.2-1.9
	Sakri	60	10	6	-	4	0.2- 1.8
	Jale	53	9	5	4	-	0.2-1.7

Table 3: Effect of AFB_1 on maize seed (Variety- A and B) Germination

Observations	Variety A (Pioneer) Concentration (µg/l)						Variety B (White maize –X08) Concentration (µg/l)					
	Control	100	250	500	1000	2000	Control	100	250	500	1000	2000
Germination index (S.E)	93 (0.06)	84 (1.28)	75 (2.07)	63 (0.46)	44 (0.54)	23 (0.63)	90 (0.04)	82 (2.05	71 (2.43	53 (0.45	39 (0.54	21 (0.73)
t- difference with control	-	9	18	30	49	70	-	8	19	37	51	69
% Inhibition	-	9.67%	19.35%	32.25%	52.11%	75.26%	-	8.63%	21.11 %	41.11 %	56.66%	76.66%

Table 4: Effect of AFB_1 on maize seeds (Pioneer) seedlings growth (Root and Shoot lengths)

Concentration AFB1 (µg/l)	Root System (cm)			Shoot system (cm)		
	Root Length	Difference with Control	% inhibition	Shoot Length	Difference with Control	% inhibition
Control	10.51±o.042	-	-	4.5±0.051	-	-
100	8.46±0.10	2.05	19.50	3.6±0.02	0.9	20.0
250	5.22±0.04	5.29	50.39	2.63±0.041	1.87	41.55
500	4.51±0.01	6	57.08	2.56±0.041	1.94	43.11
1000	3.50±0.06	7.01	66.69	1.95±0.04	2.55	56.66
2000	2.41±0.04	8.04	79.49	1.44±0.03	3.06	68

Table 5: Effect of AFB_1 on maize seeds (White maize-X08) seedlings growth (Root and Shoot lengths)

Concentration AFB1 (µg/ l)	Root System (cm)			Shoot System (cm)		
	Root length	Diff. with control	% inhibition	Shoot length	Diff with control	**%** inhibition
Control	8.78±0.10	-	-	3.5±0.8	--	--
100	7.58±0.7	1.17	13.32	2.6±0.31	0.90	25.71
250	5.5±o.o4	3.25	36.67	2.56±0.041	0.94	26.85
500	5.25±0.03	3.50	39.86	2.16±0.043	1.34	38.28
1000	3.25±0.05	5.5	62.64	1.75±0.03	1.75	50
2000	2.51±0.03	6.24	71.07	1.41±0.03	2.09	59.71

RESULTS AND DISCUSSION

The study was designed to determine the fungal diversity of two varieties of maize seeds. The experiment showed that diverse nature of fungal isolates in two varieties of maize seeds samples (var. Pioneer and White maize -X08) like *A. flavus, A. niger, Alternaria spp., Fusarium spp., Penicillium and Rhizopus* were recorded in Table -1 whereas A*. flavus* was observed in all the samples of two varieties. The prevalence of fungal infections and seed germination varied depending on maize seeds varieties and sources of seed collection. In the present investigation, various mycoflora were recorded in maize sample of variety (white maize -X08) like *Aspergillus spp., Fusarium spp. Penicillium* spp. no. of Toxigenic strains were also recorded in which aflatoxin produced by *A. flavus* i.e. $AFTB_1$, B_1B_2, B_1B_2G1. The fluctuation of mycoflora and aflatoxin production and *A. flavus* (Table 1 and 2) due to diverse agro-climate conditions for producing a variety of crops, Lack of awareness of good agricultural practices, proper storage facilities of farm, fluctuating environmental conditions during pre and post-harvest period . Above facts proved by Darbhanga is one of the district fall under north-west zone of Bihar is a flood prone regions (Diksha et al., 2022). The healthy seeds is the foundation of healthy plant a necessary condition for good yields also recorded by Mathur and Kongsdal (2003).

Table 2 showed that two varieties of (Pioneer and White maize -X08) having 189 and 181 isolates of *Aspergillus flavus* in which 59 and 37 isolates of *A. flavus* where toxigenic confirmed by SMKY liquid media. They were positive to produced AFB_1, B1B2, and B1BG1, respectively. The amount of AFB_1 was however very low, only o.2-1.8 µg/l and 0.2-1.9µg/l. The maximum aflatoxin B_1 was recorded from Kusheshwar Asthan , samples followed by Sakri and Jale .

Seed germination

The effect of five different concentrations of AFB_1 (Pioneer and White maize seed - X08) on seed germination of maize were presented in Table 3. A significant fall in seed germination was noticed at 2ppb concentration of AFB_1. The maximum inhibitions in seed germination were 9.67%, 19.35%, 32.25%, 52.11% and 75.26 % in variety Pioneer Maize seeds at 100 , 250, 500, 1000, 2000 µg/l concentration of AFB_1, respectively and in variety White maize - X08 maximum inhibition in seed germination were found to be 8.63%, 21.11%, 41.11%, 56.66%, 76.66% and 79.49% at 100, 250,500, 1000 and 2000 µg/l concentration of AFB_1, respectively.

Seedling growth

Seedling growth (root and shoot lengths) of maize seeds (Variety A- Pioneer) was drastically reduced due to the inhibitory effects of aflatoxin B_1. Percent inhibition in root length were found to be 19.50%, 57.08%, 57.08%, 66.69%, and 79.49%, respectively, and shoot length were found to be 20.0 %, 41.55%, 43.11%, 56.66% ,respectively, due to different concentrations of aflatoxin B_1 (Table 4). Seedling growth (root and shoot lengths) maize seeds (Variety B – White maize - X08) was drastically reduced due to the inhibition effects of aflatoxin B_1. The percent inhibition in root length were found to be 13.36 %, 36.67%, 39.86%, 62.64% and 71.07%, respectively. Shoot length were 25.71 %, 26.85%, 38.28%, 50% and 59.71% at 100, 250, 500, 1000, and 2000 µg/l concentration of AFB_1 , respectively (Table-5).

CONCLUSION

Seed could play an important role in the epidemics in fields for high quality and quantity so that healthy seeds of maize should be released to farmers in order to secure their outputs to return high economy.

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