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Assessment of *Andrographis paniculata* Plant Extracts to Reduce Mycotoxin Contamination in Maize Seeds

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ABSTRACT

Environmental factors like soil, temperature, and pH influence the level of contamination in cereals. In this study fungi such as *Aspergillus flavus, A. niger, Penicillium* spp., *Rhizopus* spp. and *Fusarium* spp. were recorded in all the samples of maize seeds.The range of kernel infection recorded by *A. flavus* was a maximum of 8.40% and a minimum of 4.20%. The production of aflatoxin B_1 in maize seeds was estimated in the range of 2.0 - 8.2 µg/ml. The inhibition of aflatoxin B_1 recorded was a maximum of 32.5% and a minimum of 8.5% in SMKY liquid media at 2.5 gm/ml and 0.5 gm/ml concentrations of *Andrographis paniculata* plant extracts, respectively. Similarly, the growth of *A. flavus* recorded was 47% (maximum) and 10% (minimum). The mycelial growth of *A. flavus* and Aflatoxin B_1 production were controlled by *A. paniculata,* whichis essential for the quality and quantity yield of maize seeds due to high population demands in our country

Key words: Aflatoxin B_1, Maize, Mycelium, Mycoflora, SMKY liquid medium,

INTRODUCTION

Maize (*Zea mays* L.) is the world's third leading cereal crop, followed by wheat and rice. In India, maize is used basically as a staple food and feed for livestock as well as for the manufacturing of various products. It is gradually used to produce ethanol fuel. Ethanol is mixed with gasoline to decrease the number of pollutants emitted when used to fuel motor vehicles. Environmental factors such as type of soil, rainfall, temperature, and pH values are known to influence the occurrence of molds and mycotoxin production in maize as well as in other cereals (Cotty and Jaime-Garcia, 2007; Paterson and Lima, 2010; Bernhoft *et. al*., 2012; Milani, 2013; Kebeda*et. al*., 2012; Smith *et. al*., 2016; Diksha *et. al*., 2022; Ragni and Prasad, 2023).

The contamination of food and feeds by aflatoxin B_1 and other mycotoxins continues to be of serious concern for human and animal health (Gong *et. al*., 2004; Battilani*et. al*., 2013). Mycotoxin levels fluctuate due to climatic variations such as delayed harvesting, poor drying, and storage conditions (Pitt and Miscamble, 1995; Torres *et. al*., 2014). The magnitude of mycotoxin contamination in Bihar is due to low input agronomic practices, improper grain storage, extreme weather conditions, and inadequate knowledge of action for the control of cereals (Diksha *et. al*., 2022; Ragni and Prasad., 2022; Ragni, 2023) hence the control of Mycotoxin in cereals is essential due to the high population demands of India.

Andrographis paniculata is known as "Kalmegh" found throughout tropical and subtropical Asia, Southeast Asia as well as in India. It is an erect annual herb extremely bitter in taste in all parts of the plant body. As a major active constituent, andrographolide exhibits a broad range of biological activities, such as anti-inflammatory, antimicrobial (Wiart *et. al*., 2005), antibacterial, antitumor, antidiabetic, antimalarial and hepatoprotective properties (Jarukamjorn and Nemoto, 2008). There are few reports on the antifungal properties of the crude extracts of *Andrographis paniculata* (Qurashi *et. al*., 1997; Singha *et. al*., 2003; Limsong*et. al*., 2004; Mathur *et. al*., 2011).

Therefore, an attempt has been made to record the impact of *Andrographis paniculata* plant extracts

on fungal growth and aflatoxin production in maize seeds.

MATERIALS AND METHODS

Sample collection: Maize samples of the Shankar Makka-1 hybrid variety were collected from farmers' fields in the Darbhanga district of Bihar during rainy season. *Andrographis paniculata* plant samples were collected from Gudri Herbal Market, Darbhanga, Bihar.

BGYF-Test: Aflatoxin-susceptible commercial maize seed hybrid (Shankar Makka-1) variety was chosen and its individual kernels, after natural drying in the field, were visually classified as BGYF- positive or negative. The BGYF-positive kernels showed glowing under ultraviolet light and presumably contained aflatoxin at levels greatly exceeding the regulatory limit of 20 µg/kg. BGYF positive and negative kernels (sample through *A. flavus*) were selected for testing by FTIR-PAS in a blind study (Diener and Davis, 1966).

Isolation of Mycoflora: Seed-borne mycoflora of Shankar Makka-1 var. of maize seeds were tested by using the standard blotter paper test ISTA (1999). One hundred seeds of maize samples were surface sterilized with 0.2 % NaOCl solution for 10 min,washed with distilled water and then 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 the developing colonies were examined for fungal growth. The percentage frequency of occurrence of each fungus was collected on the following formula:

$$\% \text{ frequency} = \frac{\text{No. of colonies of a particular species}}{\text{Total no. of colonies of all the species}} X\ 100$$

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

Qualitative analysis: Standard procedures for performing an analysis of aflatoxins by TLC were followed as described by Jager *et. al.*, 2013; Thomas *et. al.*, 1975. 20 g of maize sample (in powder form) was added to a 500 ml flat-bottomed flask. A mixture of 25 g hyflosupercel, 200 ml chloroform, and 20 ml distilled water was added then covered and shaken on a mechanical shaker for 30 min. After filtration (Whatman paper 1) the first 100 ml was collected. Two-thirds of a column chromatography tube was filled with chloroform after which 5 g of Na_2SO_4 was added The chloroform was drained, and the extract was transferred into the silica gel column. Hexane (100 ml) was added and then followed by 100 ml. of ethyl alcohol. Aflatoxins were eluted using 100 ml of a chloroform-methanol mixture and collected into a round-bottom flask for examination under UV light at 360 nm and compared with the standards.

Quantitative analysis: The Quantitative analysis of Aflatoxin B_1 was carried out spectroph-otometrically (Nebney and Nesbitt, 1965).

Preparation of plant extracts: Fresh plant material is collected from the study region seasonally by confirming the taxonomic identity (ethnobotanical details).The overall process may contain the following steps (Handa *et. al*., 2008).

(a) Collection of plant material and drying

(b) Size reduction

(c) Extraction

(d) Filtration

(e) Concentration

(f) Drying and reconstitution

The quality of an extract is influenced by several factors such as plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material: solvent ratio, etc. from laboratory scale to pilot scale. All the parameters are optimized and controlled during extraction. Extraction techniques separate the soluble plant metabolites through selective use of solvent.

Grain testing for fungal infestation:

Grain inspectors test random maize samples were done by exposing to bright greenish-yellow fluorescent (BGYF) light on kernels. If the Kernels are contaminated with a fungus, they turn into vivid

greenish-yellow colour (**Figure 1**). Suspicious samples are then tested further in the lab for FTIR-PAS technique (Gordon *et. al*., 1997).

Figure 1: BGYF Test

RESULTS AND DISCUSSION

A total of 5 fungal species were found to be associated under varying isolation conditions as shown in **Table 1**. The spp. of *Aspergillus flavus* and *A. niger* were recorded in all these samples where a few genera were isolated from sterilized and non-sterilized kernels. The percentage incidence of *A. flavus* was found to be maximum (40.16%) followed by *A. niger* and *Fusarium* spp. The total number of colonies recorded was 361 and 531 in surface sterilized and non-sterilized seeds, respectively. The range of kernel infection in different samples varied between 4-40%.

Aflatoxin contamination of grain is an interruption to the quality of food production and trade across the globe (Kebeda *et. al*., 2012). It also has adverse effects on the physiology of the plant system (Kumar *et. al.*, 1993). Several reports are available on how toxin causes toxicoses in animal systems (Sinha and Prasad, 1996; Sinha and Prasad, 1997., Prasad ~~G~~, 1998).Adverse effects of consumption of aflatoxin-contaminated food were linked to a large population of children experiencing poor and stunted growth in Benin (Gong *et. al.*, 2004).

Table 1: Isolation of Mycoflora on and in Maize seeds (var. Shanker Makka-1)

Sl. No.	Fungi	No. of the positive samples (58)	Total no. of colonies		% Relative infection		Range of Kernel infection (%)
			Surface Sterilized (S)	Non-sterilized (NS)	Surface Sterilized (S)	Non-sterilized (NS)	
1.	*A. flavus*	20	145	202	40.16	38.04	8.40
2.	*A. niger*	15	132	162	36.56	30.50	7.32
3.	*Penicillium* spp.	10	20	38	5.54	7.15	6.14
4.	*Rhizopus*	8	28	44	7.75	8.28	5.15
5.	*Fusarium*spp.	5	36	85	9.97	16.00	4.20
Total no. of colonies		-	361	531	-	-	-

Altogether 70 isolates of *A. flavus* representing 7 isolates from each sample were screened for their aflatoxin-producing potentialities. Out of these 35 isolates were found to be toxigenic (**Table 2**). Amongthe toxigenic strains, 25 isolates elaborated aflatoxin B_1 only, whereas 08 isolates were capable of producing aflatoxin B_1 and B_2. Only two isolates could produce B_1, B_2 and G_1. *A. flavus* isolates produced aflatoxin B_1 in the range of 2.0- 8.2 µg/ml.

Table 2 also shows that out of 20 samples of maize the natural contamination of aflatoxin 6 isolates were found to be toxigenic strains. Among these toxigenic strains 4 isolates elaborated aflatoxin B_1 production only whereas 2 isolates were capable of producing both aflatoxin B_1 and B_2. The amount of aflatoxin B_1 was, however very low i.e., only at 1.4-15 µg/kg in those contaminated samples.

Table 2: Aflatoxin-producing potentiality of *A. flavus* isolates and aflatoxin contamination in maize seed samples.

No. of *A. flavus* isolates/maize sample screened	No. of toxigenic isolates/ contaminated sample	Types of aflatoxin			Range of aflatoxin B_1 concentration
		B_1	B_1 B_2	B_1B_2 G_1	
70 Isolates	35	25	08	02	2.0- 8.2 µg/ml
20 Samples	6	04	02	-	1.4 & 15 µg/kg

The mycelium growth of *A. flavus* and aflatoxin B_1 production were recorded by different concentrations of *Andrographis paniculata* in SMKY liquid media (**Table 3**). *Andrographis paniculata* having3O- β- D- glucosyl- 14- deoxy andrographolide and 14- dedehydro andrographolide as antifungal compounds was reported to change the fungal activity in maize seeds (Sule *et. al*., 2012; Eugena *et. al*., 2015).

The minimum and maximum inhibition of growth of *A. flavus* were recorded at 10% and 47% at the concentration of plant extracts at 0.5 gm/ml and 2.5 gm/ml, respectively. Similarly, the minimum and maximum inhibition of aflatoxin B_1 production in SMKY liquid medium were 8.5% and 32.5% at 0.5 gm/ml and 2.5 gm/ml concentrations of plant extracts, respectively.

Table 3: Effect of plant extracts on Mycelium Growth and Production of Aflatoxin B_1 in SMKY liquid media

Plant extracts with different concentrations	Mycelium growth of *A. flavus* (gm ± S.E)	% inhibition	B_1 (µg/ml ± S.E)	% inhibition
Control	10 gm ± S. E	-	2 µg/ml ± S.E	-
0.5 gm/ml	9 gm ± 0.27	10 %	1.83 ± 0.007	8.5 %
1.0 gm/ml	8 gm ± 0.27	20 %	1.75 ± 0.010	12.5 %
1.5 gm/ml	7.6 gm ± 0.36	24 %	1.68 ± 0.043	16 %
2.0 gm/ml	6.3 gm ± 0.83	37 %	1.60 ± 0.006	20 %
2.5 gm/ml	5.3 gm ± 1.09	47 %	1.35 ± 0.018	32.5 %

CONCLUSION

The seasonal pattern of aflatoxin contamination of staple foods like maize in India, presents an opportunity for interventions such as biological control to reduce the growth of *A. flavus* and aflatoxin production in the rainy season through application of *Andrographis paniculata* plant extracts.It is also one of the approaches to reverse the aflatoxin inhibitory properties to enhance food production to accommodate the growing population demand in developing countries like India.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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