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Control of Toxigenic strain, *Aspergillus flavus* and Mycotoxin by the extracts of *Andrographis paniculata* in Maize seeds (*Zea mays* L.)

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Abstract
Maize is nutrient rich, staple food of the country, but it is infected with fungal toxins like Mycotoxins. Mycotoxins (Fungal toxins) are broadly divided into two major groups on the basis of mycotoxin producing fungi i.e., those fungi which invade in pre- harvest and post-harvest conditions known as storage fungi. The conditions which promote the growth of mycotoxins are moisture content, high temperature, poor hygienic conditions as well as storage and transportation. Aflatoxins one of the mycotoxins, which are carcinogenic, mutagenic, hepatotoxic, teratogenic as well as immunosuppressive, could be produced by certain strains of genus, *Aspergillus;* such as, *A. flavus* and *A. parasiticus.* In this study, we tried to know, to examine the control of toxigenic strain of *Aspergillus flavus* from maize seeds (*Zea mays* L.) through *Andrographis paniculata* plant extracts for food and health security. Comparing the effect of plant extracts obtained from *Andrographis paniculata* extracts were capable of inhibit the mycelial growth of *A. flavus* ranging from 10 - 100%. The highest and lowest levels of antifungal activity were obtained by using *A. paniculata* at various concentration *viz*, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml. Spore germination, mycelium growth of *A. flavus* was inhibited (75%) and (100%) of *A. paniculata* at 2.5 ml concentration. So, the *Andrographis paniculata* plant extracts might be used as a biological agent to decrease mycelial growth and aflatoxin production especially by *A. flavus* for protecting maize crops from this toxigenic fungus like Aflatoxins. Therefore, this plant extracts like *Andrographis paniculata* might be used as a natural preservative against biodegradation and storage contamination caused by *A. flavus* for food and health security.

Keywords: Maize seeds, *A. paniculata, A. flavus,* mycotoxin, food and health security

Introduction
Maize is an important cereal crop in many developed and developing countries of the world. It is cultivated in tropics, sub- tropics and temperate regions unto 50˚ and from sea level to 4000 m. As regards to area and production of maize (380 MT from 120 MH) followed by wheat (440 MT from 240 MH) and rice (420 MT from 140 MH), respectively. This represents 24% of the total cereal production as compared to 27% for wheat and 25% for rice. In India, U.P., Bihar, Rajasthan, Madhya Pradesh and Punjab are the safe zone for production of maize. Among the cereal crops in India, the annual production of maize is around 10 million tonnes covering 6 million hectares and its ranks 5th in area.

It is widely used for animal feed and industrial raw material in the developed countries. Maize occupies a prominent position and each part of the maize plant is put to one or the other use and nothing goes as waste.

Contamination of food products by microorganisms could lead to deterioration of the food or food products. Pathogenic fungi especially *Aspergillus flavus* are the main pathogens responsible for the alterations (Quality and Quantity) during development including post-harvest of maize seeds due to Aflatoxin produced by *A. flavus* [7].

Aflatoxins are a group of mycotoxins produced by various species of the fungal genus *Aspergillus*, particularly *A. flavus* [3, 4]. Various agricultural products might be contaminated with aflatoxin producing fungi or aflatoxins [8]. Aflatoxins have been detected in cereal grain, oil seeds, fermented beverages made from grains, milk, cheese, meat, nut products, fruit juice and numerous other agricultural commodities [2].

To investigate the control approach for the aflatoxin properties to enhance the food production to accommodate the growing population demand in developing countries like India.

Andrographis paniculata (Burm. f.) wall ex Nees belongs to *Acanthaceae* family, is an annual herbaceous plant native to India and other countries like Sri Lanka. *Andrographis paniculata* is popularly known as "King of Bitter" in English. It is also infamously called as Bhui- neem, which means "Neem of the ground" as it has a similar strong bitter taste. It is widely cultivated in Southern and South estern Asia, where it has been traditionally used to treat infections and some diseases [6, 1]. *Andrographis paniculata* extracts has interesting antimicrobial activity against *A. flavus*, *Fusarium* spp., *A. niger* and other *fungal* spp. [9, 10].

There are no cohesive reports of antifungal activity of plant extracts on aflatoxin production in maize seeds. Therefore, an attempt has been made to records the management of toxigenic strains of *A. flavus* from maize seeds through plant extracts especially *A. paniculata.*

Materials and Methods

Plant material

Plant materials comprising of leaves and stems of *Andrographis paniculata* plant were collected from Darbhanga, Gudari Market. A dried voucher sample was kept in the laboratory of Univ. Dept. of botany in L. N. Mithila University, Darbhanga.

Preparation of the plant extracts

50 gm of air-dried ground leaves and stems of *Andrographis paniculata* were submitted to hydro distillation for about 3 hrs using a clevenger apparatus [14]. The obtained plant extracts were dried over anhydrous sodium sulfate, preserved in sealed glass bottle and stored in the dark at 4 °C for further used.

Fungal culture conditions

The isolates of *Aspergillus flavus* was obtained from maize seeds. This isolate was maintained on Potato dextrose agar (PDA) media at 4 °C and sub cultured at monthly intervals.

Antifungal analysis

Inhibition of mycelial growth was using the food poison method described by [13]. The PDA plates were amended with various concentration of plant extracts. For enhancing the plant extracts solubility, 0.5% (v/v) was added. Each plate was inoculated with a mycelial plug (10 mm diameter) of *A. flavus*. All plates (triplicate) were incubated for each concentration at 28 °C for one week. Control plates with Tween- 20 was used without plant extracts. Observation of fungal growth was done at a time interval of 12 h up to one week after incubation. The mycelial growth inhibition percentage was calculated according to the following formula:

$$\text{MIC } \% = \frac{Dc-Dt}{Dc} X\ 100$$

Where, MIC= minimum inhibition concentration
Dc= mean diameter of colony in the treatment (mm).

Three replicate plates were used per treatment and the experiment was repeated three times. The MIC values were determined as the lowest concentration of plant extracts the completely prevented visible fungal growth. To determine minimum fungicidal concentration (MFC), the mycelial plugs were obtained from each petri dish treated with the plant extract concentration where no growth was observed transferred into a new plate without the plant extract and incubated at 28 °C for one week. MFC was defined as the lowest concentration at which no colony growth was observed after Sub-culturing into fresh PDA medium. Three replicates were used per treatment and the experiment was repeated three times. The plant extracts with highest level of inhibiting *A. flavus* mycelial growth were selected for the rest of experiment.

Effect of the *Andrographis paniculata* on sporulation of *A. flavus*

Effect of various concentration of extracts obtained from *Andrographis paniculata* on the ability of *A. flavus* for sporulation was evaluated on PDA medium. Various volume of *Andrographis paniculata* (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml) were mixed into each of three replicate plates of PDA. Then, the plates were inoculated with a mycelial plug (1 cm diameter) of *A. flavus*. They were incubated at 28 °C. After 7 days of incubation, 1 cm diameter mycelial plugs from a plate of *Andrographis paniculata* concentration were randomly inumersed in 5 ml distilled water in test tube, which was shaken to dislodge the spores. The number of spores from the replicates of each concentration were counted using hemocytometer. The percentage of sporulation inhibition was determined using the following formula:

$$\text{Inhibition of sporulation (\%)} = \frac{Nc-Nt}{Nc} X\ 100$$

Where, Nc = Number of fungal spores in control
Nt = Number of fungal spores in treatment [11, 12].

Effect of the *Andrographis paniculata* extracts on conidia germination of *A. flavus*

Conidia of *A. flavus* cultured on PDA plates were taken and conidial suspension (105 spore/ml) were prepared. Different concentration of *Andrographis paniculata* (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml) were mixed in PDA plates. One ml of conidial suspension was spread in each PDA plate containing the plant extracts and they were incubated at 28 °C for one week. Germination of spores was investigated with stereomicroscope and inhibition of spore germination was determined using the following formula:

$$\text{Inhibition of spore germination (\%)} = \frac{Nc-Nt}{Nc} X\ 100$$

Where, Nc is the number of fungal colonies in control and Nt is the number of fungal colonies in treatment [5].

Results and Discussion

Antifungal analysis

A preliminary screening showed Bitter plant extracts like *Andrographis Paniculata* exhibited various degrees of antifungal effect in Table-1. Extracts tested were capable of inhibit the mycelial growth of *Aspergillus flavus* ranging from 10 to 75%. The lowest and highest levels of mycelial growth inhibition were obtained using the plant extracts of *Andrographis paniculata*. The plant extract of *Andrographis paniculata*, which showed the highest inhibitory growth effect on *Aspergillus flavus* at 2.5 ml concentration.

Table 1: Bitter plant used in the preliminary Screening for antifungal activity

Bitter plant	Common Name	Family	Antifungal compound
Andrographis paniculata (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml)	King of Bitter, green chiretta	Acanthaceae	Methanol

Sporulation of *A. flavus*

The results of counted spores in Inhibitory concentration of *Andrographis paniculata* plant extracts compared to control revealed that the plant extracts were more or less significantly effective on sporulation of *A. flavus* at the used concentration (Table- 2 and Fig- A).

Table 2: Effect of different concentration of *Andrographis paniculata* on *A. flavus* in PDA medium (Petri plates).

	***Andrographis paniculata* with different concentration**				
Concentration	**0.5 ml**	**1.0 ml**	**1.5 ml**	**2.0 ml**	**2.5 ml**
Control	**7th day**	**7th day**	**7th day**	**7th day**	**7th day**
Isolates of *A. flavus* from maize seeds	1.8	1.5	1.2	1.0	0.5
Differences with control	0.2	0.5	0.8	1	1.5
% inhibition	10%	25%	40%	50%	75%

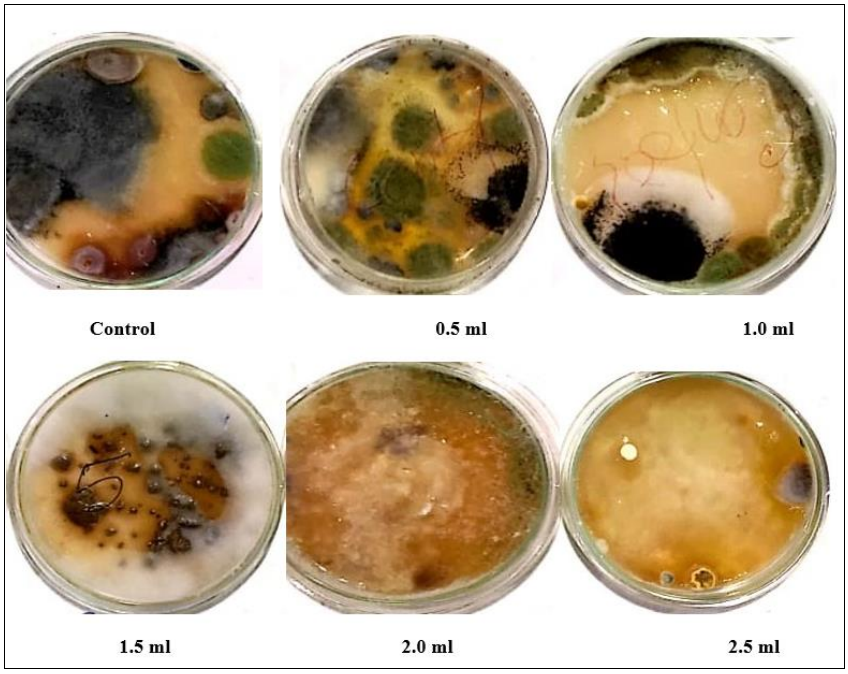

Fig 1: A, Patri plates showing different concentration of *Andrographis paniculata* on *A. flavus* in PDA medium.

Conidial germination of *A. flavus*

Germination of *A. flavus* spores was completely inhibited by all concentration of *Andrographis paniculata* extracts compared to control in which spore generating occurred about 24 h after inoculation (Table-3).

Table 3: Percentage (%) inhibition of Sporulation and Spore germination in PDA medium.

Concentration of Plant extracts	**Inhibition of Sporulation (%)**	**Inhibition of Spore germination (%)**
Control	0	0
IC	0	75%
MIC	0	100%

Where, IC= Inhibitory concentration and MIC= Minimum inhibitory concentration.

Discussion

The mycoflora of the tested cereal grains including, maize, wheat, peanut presented different fungi, with different frequencies and percentages, some with the ability to produce aflatoxins. A total of 15 species of fungi belonging to 8 genera were isolated and the greater number of species was related to the genus *Aspergillus; A. flavus* being the most dominant.

In this study antifungal capability of the plant extracts obtained from *Andrographis paniculata* against *A. flavus* was investigated. Our data showed that used of plant extracts in this study had inhibitory effect on mycelial growth of *A. flavus*. The *Andrographis paniculata* extracts had complete inhibition. The plant extracts of *Andrographis paniculata* revealed the highest level of inhibitory effect on mycelial growth of *A. flavus* among *Andrographis paniculata* plant extracts tested. So, it was selected to be used in the rest of experiments on analysis of its antifungal activities against *A. flavus*. Our findings also showed that spore germination of *A. flavus* was more or less inhibited by Inhibitory concentration (IC) and Minimum inhibitory concentration (MIC) of *A. Paniculata* plant extracts compared to control. Therefore, these Bitter plant extracts like *A. paniculata* is capable of controlling a wide range of fungi.

Conclusion

Our research findings showed that, the high level of antifungal capability of *Andrographis paniculata* plant extracts have recorded against *A. flavus* and aflatoxin production. Therefore, this plant extracts might be used as a natural preservative instead of synthetic chemical fungicides in food and agricultural products against biodegradation and storage contamination caused by *A. flavus* for food and health security.

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Conflict of interest

The author declare that they have no conflict of interest.

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