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Efficacy of *Cassia alata* Leaves Powder on Inhibition of *Aspergillus flavus* Growth and Aflatoxin Production

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Aflatoxin, a toxin produced by the fungus *Aspergillus flavus* Link: Fries, occurs naturally in maize (Zea mays L.). Aflatoxin is a potent human carcinogen and is also toxic to livestock, pets, and wildlife. When contaminated with aflatoxin, the value of maize grain is markedly reduced. In the present study, an attempt was made to evaluate the inhibition of *Aspergillus flavus* growth and aflatoxin production with using *Cassia alata*. The experimental design was elevated using T1 (control, Healthy maize), T2 (Healthy maize+*Aspergillus flavus*, T3 (Healthy maize + *Aspergillus flavus* + Plant powder), T4 (Healthy maize + Plant powder). The healthy samples were inoculated artificially with *A. flavus* and treated maize seeds were stored at room temperature in airtight polyethylene bags till analysis. This stored maize was taken on 3rd, 6th, 9th and 12th (3 month interval) month after treatment for analysis of aflatoxin production and growth of *Aspergillus flavus*. Isolated colonies of *A. flavus* was identified based on the cultural, morphological and biochemical characteristics. Further, 10% concentration the *Cassia alata* leaves powder delayed the growth of *A. ûavus* and complete inhibition was observed upto 120 days. The aflatoxin production was observed in HPTLC and the highest percentage reduction inT_3 compare other treatments. Hence, the present study was concluded that the antifungal effects of the plant powder observed and has a potential for use as a aflatoxin inhibitor.

Keywords: Aflatoxin, *Aspergillus flavus,Cassia alata*, Maize, HPTLC.

India is a country of vast population and about 70% of people are engaged in agriculture. Rapid population growth in the developing countries leads to an ever-increasing demand for food. Because of rising demand, production has steadily increased over the past years. In the production of food crops losses occur during the growth cycle in the field. Further losses occur during harvest, where up to 5% of grain weight can reduce the agricultural output[1]. Further losses occur during storage, where found average damages of 30% in stored maize after six months storage in Togo[2]. Storage pests are the main cause of these losses, and under the tropical climatic conditions the development of storage fungi, especially species of the genera *Aspergillus spp*. and it produced toxin, known as aflatoxin.

Aspergillus flavus is a soil-inhabiting, filamentous fungus that saprophytically utilizes a wide range of organic substrates. Though *A. flavus* is considered a saprophyte, it is also an opportunistic pathogen[3] that can invade

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agronomically important oil seed crops such as corn, peanut and cottonseed that are under biotic or abiotic stress. Among the toxic secondary metabolites produced by this organism are the aflatoxins (AFs) with aflatoxin B1 being the most potent natural carcinogen known[4]. The foods at highest risk of aflatoxin contamination are corn, peanut and cotton seed[5]. Aflatoxin B1 has been detected in 80% of maize samples obtained from different locations in Southeast Nigeria[6] and similarly 92 % of animal feed samples taken from commercial sources in Thailand were contaminated with aflatoxin B1[7]. In at least three parts of the world, East Africa, the Philippines and Thailand, good epidemiological evidence has been collected showing a correlation between the incidence of liver cancer and exposure to aflatoxins[5].

Aflatoxins have also been identified as a potential biological weapon for food and water contamination[8]. Physical, chemical and biological methods have been investigated in order to prevent the growth of aflatoxin producing fungi and to eliminate or reduce the levels of aflatoxins or to degrade or detoxify aflatoxins in foods and feeds[9]. Control by naturally produced agents is becoming increasingly important because of consumers' mistrust of food and feed treatments that involve using synthetic xenobiotic substances. Natural plant compounds have been used traditionally to preserve foods in countries like Japan, India and Russia[10]. Extracts and powders of various spices, herbs and essential oils have been reported to have antimicrobial activity against aflatoxin producing fungi and some of them also inhibit aflatoxin formation[11-12]. Aim of the present study was to evaluate the inhibition of *Aspergillus flavus* growth and aflatoxin production with using *Cassia alata* and has a potential for use as a aflatoxin inhibitor.

MATERIALS AND METHODS

Sample collection

The healthy and contaminated maize (*Zea mays* L.) seed samples were collected from storage facilities at Thanjavur District, Tamilnadu, India. Samples were placed directly in polyethylene bags and transferred immediately to the laboratory where they were stored in a cool place for further investigation. The contaminated maize seed sample was used as isolation of aflatoxin producing fungi.

Isolation and identification of aflatoxin producing fungi

The serial dilution procedures were followed. The potato dextrose agar was prepared and it was poured onto the sterile Petri plates. After solidification, the selected dilution factors 10^{-2} to 10^{-4} were spread on the medium respectively. Then the plates were incubated. The isolated fungal strain was identified based on their cultural and morphological characteristics.

Collection of plant and preparation of powder

The plant of *Cassia alata* (leaves) was collected from wild area of Thanjavur District, Tamilnadu, India. The leaves of *Cassia alata* was air dried and crushed to small piece using Mortar and Pestle and powdered in an electric grinder.

Experimental Design

T_1 - Control (Healthy maize)
T_2 - Healthy maize + *Aspergillus flavus*
T_3 - Healthy maize + *Aspergillus flavus* + Plant powder
T_4 - Healthy maize + Plant powder

The healthy samples were inoculated artificially with *A. flavus* as per the method[13]. The treated maize seeds were stored at room temperature in airtight polyethylene bags till analysis[14]. This stored maize was taken on 3rd, 6th, 9th and 12th (3 month interval) month after treatment for aflatoxin analysis and growth of *Aspergillus flavus.*

Fungal spore

Sample, 1 g from each flask was serially diluted 10-fold with sterile distilled water and 0.1 ml of each dilution was spread uniformly on potato dextrose agar plates and incubated in the dark for 3 days. On the 3rd day, colonies were counted and expressed as log colony forming units per gram (log CFU/g).

Estimation of Aflatoxins by High Performance Thin Layer Chromatography (HPTLC)

Aflatoxin determination was based on the Association of Official and Analytical Chemists (AOAC) BF method (AOAC, 2001) with some modifications. The experimental design treated maize samples (25g) were blended with 200ml Acetone: water (85 + 15, V/V), 2g NaCl and 5g Celite powder and mixed. Mixture was allowed for half an hour and then filtered. 25ml of hexane was added 2 times and the lower layer was separated and put into a free test tube. Lead acetate 20% in 0.3%

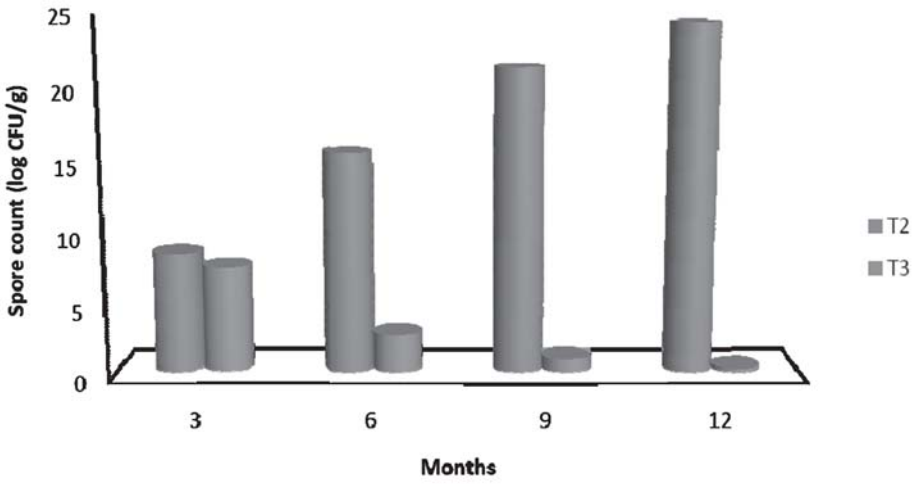

Fig 1. Comparison of spore count between T_2 and T_3 Treatments

acetic acid was added (Added 1 ml/10ml of extract) and allowed for setting for 15 min and then filtered.

Filtrate was collected in a separating funnel. 50 ml of chloroform was added and shaken for 2 min. Lower layer was removed in anhydrous sodium sulphate. Extract was evaporated in water bath and the final layer of solution was transferred to the tube containing chloroform of about 2 ml. Samples were injected into HPTLC and florescence intensity was calculate under long UV lamp at 365nm wavelength.

Calculation

$$\text{Concentration of Aflatoxin in } \mu g/kg = \frac{S \times Y \times V}{X \times W}$$

Where,

S = µl aflatoxin standard which matches the unknown

Y= Concentration of Aflatoxin standard µg/ml.

RESULTS AND DISCUSSION

In this present study fungal species was isolated from the contaminated maize (*Zea mays* L.) seed samples. Number of fungal colonies was isolated from the soil samples. Among these isolated colonies of *A. flavus* was identified based on the cultural, morphological and biochemical characteristics. The results were presented in the Fig -1. The *A. flavus* as a major fungal contaminant in maize seeds and humans are exposed to its spores every day without any clinical outcome. But, in some cases when persons are exposed to intense spore dust, hypersensitivity reactions and lung disease (primary Aspergillus pneumonia, aspergilloma, allergic bronchopulmonary aspergillosis, and invasive Aspergillus) may be observed[15]. Many researchers have reported *A. flavus* as a dominant mycoflora from different substrates[16].

The dried *Cassia alata* leaves powder was used for inhibition of aflatoxin production and growth of *A. flavus*. The effect of *Cassia alata* leaves powder on the growth of *A. ûavus* and production is presented in Table – 1. An inhibitory effect on the *A. ûavus* growth production was observed at all concentrations of the leaves powder sample used in the study and was found to be dose dependent. The pattern of inhibition shows that at lower concentration of 1% inhibition of *A. flavus* is relatively greater than inhibition of growth of the fungus. 10% concentration the *Cassia alata* leaves powder delayed the growth of *A. ûavus* and complete inhibition was observed upto 120 days.

Efficacy of *Cassia alata* leaves powder to prevent aflatoxin production in maize was estimated to all treatments. The investigated results were presented in Table 2. The highest percentage reduction in aflatoxin production was observed in T_3 compare than other treatments. The percentage reduction in aflatoxin production (%) every three months increased in T_3. Similarly T_2

Table 1. Effect of certain Cassia alata leaves powder to prevent Aspergillus flavus growth

S. No.	Compounds	Concentration	Spore count (log CFU/g)	Inhibition (%)
1	Control	-	24±2.26	-
2	C. alata	1%	16±1.85	36
		2%	8±0.33	68
		5%	4±1.15	84
		10%	0	100

Mean ± Standard Deviation;

Table 2. Efficacy of Cassia alata leaves powder to prevent aflatoxin production in maize

S. No	Months	Treatments	Spore count (log CFU/g)	Aflatoxin (ppb)	Percentage reduction in aflatoxin production (%)
1	3	T1	ND	ND	-
		T2	8.44±0.012	10842±118.22	-
		T3	7.42±0.022	7852±122.45	27.5
		T4	ND	ND	-
2	6	T1	ND	ND	-
		T2	15.51±0.016	19953±124.62	-
		T3	2.67±0.032	554±8.72	97.22
		T4	ND	ND	-
3	9	T1	ND	ND	-
		T2	21.35±0.056	23782±137.43	-
		T3	0.90±0.008	160±4.66	99.32
		T4	ND	ND	-
4	12	T1	ND	ND	-
		T2	25.44±0.072	24658±120.62	-
		T3	0.22±0.004	88±2.12	99.64
		T4	ND	ND	-

Mean ± Standard Deviation; ND - not detected

the spore count (log CFU/g) and (ppb) Aflatoxin were equally increased. The Fig – 1 illustrates T_3 and T_2 treatments were increased on opposite direction. The fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by several workers[17-19]. Therefore, the present study is an important step toward development of plant based pesticides, which are eco-friendly for the management of storage fungi, today and in near future.

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