AFLATOXINS IN EGYPTIAN SORGHUM GRAINS: DETECTION AND ESTIMATION

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Abstract

Nine fungi were isolated from naturally infected grains of all sorghum varieties used in this study. Out of these fungi, two namely; *Aspergilus flavus* and *A.parasiticus* are well known to produce aflatoxins. The aflatoxin B1 was detected in the grains of samples obtained from 6 sorghum varieties ranging from traces to 11.2 ug/kg grains, while the aflatoxin B2 was found in grains of 3 sorghum varieties in concentrations ranging from 3.4 to 11.2 ug/kg grains. On the other hand, the aflatoxins G1 and G2 were not detected in the naturally infected grains of all sorghum varieties tested .

Following artificial inoculation with *A.flavus* and *A.parasiticus*, individually, in plants of cv. Giza 15, the four aflatoxins B1, B2, G1, and G2 were produced in the grains with high concentration, when compared with aflatoxins B1 and B2 produced in the naturally infected grains. Grains inoculated with *A.parasiticus* produced the highest amount of aflatoxin G1(50 ug/kg), while grains inoculated with *A.flavus* produced the highest amount of the aflatoxins B1, B2 and G2 (maximum 20,20 and 25 ug/kg, respectively).

INTRODUCTION

Aflatoxins are secondary metabolites produced by certain toxin producing strains of several fungi, especially *Aspergillus flavus* and *A.parasiticus*, grown on suitable substrates when environmental conditions, moisture and temperature, are suitable for toxin production. Aflatoxins have been recognized as potential toxins, contaminating agricultural crops and their products worldwide. Aflatoxins have adverse effect on people and animal (Heathcote and Hibbert, 1978). Maize grains and

its products are most frequently contaminated with aflatoxins that can, when present in high levels, cause significant human and animal diseases (Christensen *et al.*, 1973).

The most important factors affecting fungal growth and aflatoxins productions are moisture content and temperature; but drying and cooling of grains are the principal means of control (Sauere and Tuite, 1986) Aflatoxins are considered as potential threat to food safety and human health (Goldblatt, 1969). In 1969 Food and Drug Administration (FDA) in the USA stated that, the safe concentration of aflatoxins in food commodities must be below 20 ug/kg. In 1995, the Egyptian Organization for Standardization and Quality Control also adopted the same concentration as a safe limit.

This investigation was carried out to detect the four kinds of aflatoxins B1, B2, G1 and G2 and estimate the level of contamination in naturally and artificially infected sorghum grains.

MATERIALS AND METHODS

Sampling :

Eight samples obtained at random from 8 sorghum varieties and lines cultivated at Giza Res. Station were used in this investigation (Table 1). The sorghum varieties and lines were naturally infected with different fungi. Three more samples were obtained from plants of variety Giza -15 inoculated artificially with *A.parasiticus*. The inoculation was made in the boot of the plants at milky stage before emergence of the heads (65 days from planting).

Isolation:

Naturally infected heads were obtained from different sorghum lines. The grains of each line were mixed and samples of them were used for isolating the seed-borne fungi. Isolation was carried out using blotter method described by Neergaard (1979). Four handered grains from each sample were tested. Sorghum grains were treated with 3% sodium hypochlerite for 2 min. Twenty five grains were plated in each Petri-dish containing three wet adsorbent blotter papers. The plates were incubated at 20^{o}C for 7 days, then examined under stereo and ordinary microscopes. The isolated fungi were identified according to Alexopoulos and Mims (1979), Ellis (1976) and Booth (1971). Out of the isolated fungi, two aflatoxin -

Table 1. Frequency (%) of different seed-borne fungi associated with naturally infected grains of eight sorghum varieties and lines.

Sorghum varieties	*A. flavus**	*A.parasiticus**	*F.graminearum*	*F.moniliforme*	*Cephalosporium acremonium*	*Penicillium sp.*	*Nigrospora sp.*	*Alternaria sp.*	*Curvularia sp.*
BT x 623	20.4	12.6	10.6	9.3	6.7	11.5	8.7	1.2	9.0
BT x 631	17.2	8.8	9.5	11.5	10.6	12.5	7.6	12.3	10.0
ICSB-14	19.4	11.4	15.6	14.9	18.5	8.6	11.0	10.6	11.0
Dorado	14.3	8.5	12.3	11.2	6.9	5.8	10.5	18.4	12.1
Local 129	21.2	11.7	12.4	14.3	9.6	8.7	9.5	12.6	0.0
Giza 123	19.4	18.9	16.7	10.3	8.7	7.9	0.0	11.5	6.9
Tam 334	24.3	15.9	13.6	10.6	9.3	8.9	7.0	10.4	0.0
Giza 15	20.3	16.2	14.3	12.6	9.6	11.7	5.6	9.7	0.0

* Aflatoxin producing fungus.

producing fungi namely; *A.flavus* isolates 1 and 2, and *A.parasiticus* were selected for artificial inoculation.

Artificial inoculation:

Each fungus was inoculated in ten plants of Giza - 15 variety. The spore suspension of each fungus was prepared from 7 days old cultures grown on Potato Dextrose Agar (PDA) medium and incubated at 27-28°C. Spores of each culture were collected from 4 Petri-dishes in 250 ml sterile water. The concentration was adjusted to $1x10^7$ spores/ml for each fungus. Five mlliliters of the spore suspension were injected into the boot of the plant before the head emergence at milky stage (65 days from planting). Estimation of aflatoxins in the grains was carried out at harvest. The two fungi *A.flavus*-1 and 2, and *A.parasiticus* were reisolated from inoculated plants.

Extraction, identification and determination of aflatoxins:

The method adopted by Singh *et al.*, (1991) for aflatoxins extraction, identification and estimation was used. Aflatoxins concentration in the sample (ug/kg) was calculated according to the formula:

$$\frac{S \times Y \times V}{W \times Z}$$

Where :
S = volume of aflatoxin standard, in ul of equivalent intensity of the sample.
Y = concentration of aflatoxin standard in ug/ml.
V = volume of solvent required to dilute final extract in ul.
Z = volume of sample extract in ul required to give fluorescence intensity comparable to that of S ul of standard.
W = weight of original sample in gram contained in the final extract.

RESULTS AND DISCUSSION

Isolated fungi:

Obtained results as presented in Table (1) show that, nine seed borne fungi were isolated from the naturally infected grains of sorghum. Out of these, the two fungi *A.flavus* and *A.parasiticus* are well known to produce aflatoxins. *A.flavus*, however, was predominant in the grains, than other aflatoxin - producing fungi. The other associated fungi were not reported, in the available literature, to produce aflatoxins, but this does not mean that they can not produce other mycotoxins. Moreover, these fungi could affect or inhibit aflatoxin production when found in association with aflatoxin-producing fungi (Choudhary, 1992).

Aflatoxin in sorghum grains naturally infected with fungi :

The aflatoxins were estimated at harvest in the grains of eight sorghum varieties naturally infected with the two aflatoxin - producing fungi *A.flavus*, *A.parasiticus* and some other associated fungi using the method adopted by Singh *et al.* (1991).

Table 2. Concentration of aflatoxins in sorghum grains naturally infected with *A.flavus*, *A.parasiticus* and some other associated fungi.

Sorghum varieties		Aflatoxin ug/kg sorghum grains			
		B1	B2	B1	B2
1	BT x 623	0.0	0.0	0.0	0.0
2	BT x 631	4.7	0.0	0.0	0.0
3	ICSB-14	traces	0.0	0.0	0.0
4	Dorado	5.9	0.0	0.0	0.0
5	Local 129	0.0	0.0	0.0	0.0
6	Giza 123	11.2	11.2	0.0	0.0
7	Tam 334	6.8	10.2	0.0	0.0
8	Giza 15	3.4	3.4	0.0	0.0

Data summarized in Table (2) show that grains of sorghum varieties BT x 623 and Local 129 were completely free from aflatoxins, although aflatoxin-producing fungi were detected in their grains. This might be due to the interference or reaction of other associated fungi. Such results were previously observed by several investigators. Nichols (1986) stated that not all isolates of *A.flavus* produce aflatoxins and that the presence of *A.flavus* in grains does not necessarily mean that aflatoxins will be present. Also, Maing *et al.* (1973) stated that associated fungi may suppress aflatoxin production which is less produced by *A.flavus* when grown in mixed cultures than grown in pure culture. Bullerman *et al.* (1984) found that aflatoxins production was inhanced in the presence of certain amino acids, fatty acids and Zinc. Moreover, the aflatoxins G1 and G2 were not detected in the grains of all tested sorghum varieties. This might be attributed mainly to the unsuitability or lack of favorable conditions, especially temperature and humidity, under which these aflatoxins are produced.

The concentration of the aflatoxin B1 in the grains of the other sorghum varieties ranged between traces and 11.2 ug/kg grains. Aflatoxin B2 on the other hand, was detected only in the grains of three sorghum varieties, ranging between 3.4 and 11.2 ug/kg grains. Several factors affecting aflatoxin production i.e., carbohydrate

content, mineral content and the presence of inhibitory agents (Jarrar and Natour, 1984). The unsuitability of some varieties for aflatoxin production might be one of these factors. The highest concentrations of aflatoxins B1 and B2 found in the grains of the variety Giza 123 (11.2 ug/kg grains), however, is still in the safe limit adopted by FDA of the USA (20 ug/kg grains). In this connection, Gorman and Kang (1991) mentioned that, a review of literature shows that maize varieties exhibit different levels of susceptibility to the attack of storage fungi, this suggests the possibility of a similar response in the aflatoxin production.

Concentration of aflatoxins in artificially infected sorghum grains

The aflatoxins B1,B2, G1, and G2 were estimated at harvest in the grains of the sorghum variety Giza-15 artificially inoculated with the 3 isolates of aflatoxin producing fungi. *A.flavus*-I and-2, and *A.Parasiticus*.

Table 3. Aflatoxin production in the grains of sorghum Giza-15 variety inoculated with 3 aflatoxin producing fungal isolates.

Aflatoxin producing fungi	Aflatoxin ug/kg sorghum grains			
	B1	B2	B1	B2
A.flavus, isolate-1	10.0	10.0	13.3	10.0
A.flavus, isolate-2	20.0	20.0	25.0	25.0
A.parasiticus	15.0	13.3	50.0	20.0
control	0.0	0.0	0.0	0.0

The results presented in Table (3) show that the concentrations of aflatoxin produced by the three fungal isolates *A.flavus* 1 and 2, and *A.Parasiticus* in the grains of sorghum variety, Giza-15, following artificial inoculation were much higher than those produced by the same fungi under natural infection. These results were expected because of the high colonization of the grains by the two fungi after the artificial inoculation. Some of these concentrations, however, exceeded the safe limit adopted by the FDA of the USA (20 ug/kg). The fungus *A.parasiticus* produced the highest amount of aflatoxin G1 compared with the other fungi. Wogan and Newbrene (1967) stated that, the toxicity of aflatoxin B1 and G1 is higher than that of aflatoxins B2 and G2. *A.flavus* 2, on the other hand, produced the highest amounts of aflatoxins B1, B2 and G2. Zein El-Abedeen *et al.*, (1994) found that, aflatoxin produc-

tion in the artificially inoculated maize grains with *A.flavus*, *A.parasiticus* was higher than in naturally infected ones, Guo *et al.* (1993) found that maize varieties varied in level of aflatoxin production under artificial inoculation with the fungus *A.flavus*. Data also show that the two isolates of *A.flavus* produced the four kinds of aflatoxins, but the amounts produced by isolate No. 2 were almost as double as the amounts produced by isolate No 1. In this connection, Fahim *et al.* (1983) tested isolates of A.flavus for aflatoxin production. They found 5 of them producing aflatoxin B1, and the highest concentration (40 ppm) was produced by the isolate Fayoum-1.

This clearly show the different potentials of isolates of toxin - producing fungi to produce such toxins both in type and quantity. It also shows that it is necessary to detect the toxin and not only the fungus.

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سموم الأفلاتوكسينات في حبوب الذرة الرفيعة المصرية، اكتشافها وتقديرها

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تم عزل ٩ أنواع من الفطريات من حبوب الذرة الرفيعة المصابة طبيعيا وذلك من أصناف الذرة الرفيعة التى استخدمت فى هذه الدراسة. ومن ضمن هذه الفطريات يوجد الفطرين *Aspergillus flavus* & *A.Parasiticus* وهما من الفطريات المعروفة بانتاجها للأفلاتوكسينات. وقد وجد الأفلاتوكسين B1 فى العينات المأخوذة من ٦ أصناف مختلفة من الذرة الرفيعة بتركيز يتراوح ما بين آثار و ١١,٢ ميكروجرام/كجم حبوب، بينما وجد الأفلاتوكسين B2 فى العينات المأخوذة من ٣ أصناف مختلفة من الذرة الرفيعة وبتركيز يتراوح ما بين ٤،٢ الى ١١,٢ ميكروجرام/كجم حبوب. ومن ناحية أخرى اتضح عدم وجود الأفلاتوكسينات G1 , G2 فى نفس حبوب الذرة الرفيعة المصابة طبيعيا.

تم إجراء عدوى صناعية لنباتات الذرة الرفيعة جيزة ١٥ بكل من الفطرين *A.parasiticus & A.flavus* وبتقدير الأفلاتوكسينات الموجودة فى حبوب الذرة الرفيعة بعد إجراء العدوى الصناعية تم اكتشاف وجود الأنواع الأربعة من الأفلاتوكسينات G1, G2,B1 و G2 وبتركيز أعلى من تركيز الأفلاتوكسينات B2,B1 والتى وجدت فى الحبوب المصابة طبيعيا.

كما وجد أن حبوب الذرة الرفيعة التى لقحت نباتاتها بالفطر *A.parasiticus* قد أنتجت أعلى تركيز من الأفلاتوكسين G1 بينما الحبوب التى لقحت بالفطر *A.flavus* أنتجت أعلى تركيز من الأفلاتوكسينات G2,B2,B1.