Effects of Varied Substrates on Aflatoxin Production by *A. parasiticus*

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Sterilized and nonsterilized wheat kernels, soybean seeds, sesame seeds, peanut and faba bean were infected by *A. parasiticus*. The chemical composition, aflatoxin content and fatty acid patterns of the seeds were determined. The aflatoxins B_1, B_2, G_1 and G_2 were detected, and the amounts of the unsaturated toxins (B_1 and G_1) were greater than the respective dihydro derivatives (B_2 and G_2). Sterilized seeds infected by the fungus contained greater amounts of aflatoxins than those infected without previous sterilization. The highest and lowest toxicity indices were recorded for sterilized wheat and soybeans, respectively. Sesame, peanut and soybean exhibited intermediate toxicity indices. The toxicity of the aflatoxins produced was related significantly in every instance to the carbohydrate and lipid:protein ratio, and not to the polyunsaturated fatty acids of the seeds.

Aflatoxins are known to be produced, as secondary metabolites, by two closely related *Aspergillus* species, *A. flavus* and *A. parasiticus* (1). Aflatoxins have been found in many commodities such as groundnuts and groundnut products (2-4), cottonseeds and cottonseed products (3-5), other oilseeds and their products (6), and in maize and some other cereal grains (7,8). In fact, almost all foods are susceptible to mold growth during some stage of production, processing, storage or transport. The presence of aflatoxins in a particular commodity usually is associated with the high moisture content of the product and unsuitable storage conditions (9).

Aflatoxin contamination of several products is a major problem throughout the world. These mycotoxins are hepatocarcinogens, mutagens, teratogens and toxins (10,11). The present study was undertaken to determine the distribution of aflatoxins in some cereal seeds which differ greatly in their chemical compositions.

MATERIALS AND METHODS

Seeds and grains. Wheat kernels (*Triticum aestivum* L., Giza 157 variety); soybean (*Glycine max*, Calland variety); sesame seeds (*Sesamum indicum* L., Giza 125 variety); peanuts (*Arachis hypogea* L., Giza 4 variety), and faba bean (*Vicia faba* L., Giza 3 variety) were obtained from the Agriculture Research Center, Giza, Egypt.

Fungus. Aspergillus parasiticus (ATCC 120920) was obtained from the Tropical Product Institute, London, England. This strain was checked for purity and identity (1).

Preparation of spore suspension. A. parasiticus was grown on a potato-dextrose-agar (Difco) slant for 10 days at 28 C. Spores were harvested by adding sterilized Tween 80 solution (0.01%, v/v); filtered through several layers of sterilized cheesecloth; centrifuged; washed three times with sterilized, distilled water, and suspended in sterilized Tween 80 solution (0.01%, v/v). The number of conidia was estimated by the plate count, and the suspension was adjusted to contain approximately 10^6 spore/ml.

Culture conditions. A portion (50 g) of sterilized and nonsterilized peanut, wheat kernels, soybean, sesame seeds and faba bean was placed in a series of 250-ml Erlenmeyer flasks. Distilled water was added to each flask until the seed moisture content rose to 30%. The amount of water added was calculated theoretically on the basis of the original moisture content of the seeds. The flasks were kept at 5 C for 24 hr, and each flask was inoculated with 1ml of the spore suspension (10^6 spore/ml). Incubation was conducted at room temperature (28 C ± 2 C) for 6 days as stationary cultures.

Extraction of aflatoxins. The aflatoxins of the seeds and kernels under study were extracted by blending them with 5-fold acetone-water (80:20, v/v), followed by removal of the contaminants (12).

Qualitative and quantitative determination of aflatoxins. An aliquot (2 ml) of the chloroform extract was introduced into a mini-column packed from the bottom to the top with glass wool, drierite (8-10 mm), florisil (8-10 mm), silica gel (16-20 mm), neutral alumina (8-10 mm), drierite (8-10 mm) and glass wool. After elution of the column with 3 ml chloroform-acetone (9:1, v/v), it was viewed under UV light (365 nm). Aflatoxins were then eluted with chloroform and fractionated by thin layer chromatography using precoated plates with silica gel 60 (0.2 mm thickness). Plates were developed at room temperature using a mixture of chloroform:acetone (9:1, v/v) and viewed under 365 nm UV. Aflatoxins were measured by comparing the fluorescence of the unknown to four standards (B_1, B_2, G_1 and G_2) obtained from the Tropical Product Institute, London, England (12).

General chemical analysis. Moisture, ash, total proteins, lipids and carbohydrate content were determined according to A.O.A.C. methods (13).

Lipid extraction. The lipids were extracted using $CHCl_3$:MeOH (2:1,v/v) (14).

Preparation of fatty acids. The lipid materials were saponified, unsaponifiable matter was removed and the fatty acids were collected. The free fatty acids were methylated with diazomethane (15).

Determination of fatty acids. The fatty acid methyl esters obtained from kernels or seeds were analyzed by a GCV Pye Unicam gas liquid chromatograph equipped with dual flame ionization detectors. The separation conditions were exactly as reported by Farag et al. (16). Results are expressed as an area percentage of the chromatograms.

Statistical analysis. The relationship between aflatoxin production and the total carbohydrate plus lipid:

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TABLE 1

Aflatoxin Pattern of Some Nonsterilized and Sterilized Grains or Seeds Infected by *A. parasiticus*

Commodity	Aflatoxin concentration (ppm)				Toxicity[a] index	Visible growth
	B_1	B_2	G_1	G_2		
Nonsterilized substrates						
Peanut	175.0	12.0	121.0	13.0	235.0	1 +
Faba bean	35.0	4.0	32.0	4.0	51.0	3 +
Soybean	117.0	8.0	323.0	25.0	270.0	4 +
Wheat	117.0	2.0	81.0	2.0	155.0	2 +
Sesame	47.0	1.0	16.0	1.0	54.0	2 +
Sterilized substrates						
Peanut	468.0	40.0	404.0	8.0	663.0	2 +
Faba bean	351.0	60.0	323.0	105.0	527.0	1 ±
Soybean	292.0	40.0	364.0	63.0	477.0	3 +
Wheat	877.0	80.0	882.0	126.0	1318.0	1 +
Sesame	526.0	50.0	323.0	8.0	686.0	1 +

[a]Toxicity index refers to the sum of the actual toxic amounts of the 4 aflatoxins when calculated as B_1.

TABLE 2

The Chemical Composition (%) of Peanut, Faba Bean, Soybean, Wheat and Sesame

Commodity	Proteins N × 6.25	Lipids	Total Carbohydrates	Ash	C + L:P ratio	Toxicity index[a]
Peanut	26.16	44.61	20.81	2.17	2.5:1.0	633
Faba bean	30.61	1.10	51.04	5.77	1.7:1.0	527
Soybean	35.17	23.66	29.90	6.17	1.5:1.0	477
Wheat	14.47	2.27	75.50	3.97	5.4:1.0	1318
Sesame	22.10	58.30	14.20	3.25	3.3:1.0	686

[a]Toxicity index refers to the sum of the actual toxic amounts of the 4 aflatoxins when calculated as B_1.

protein ratios of the seeds under study was statistically analyzed using a correlation coefficient (r) (17).

RESULTS AND DISCUSSION

Table 1 shows the aflatoxin distribution of sterilized and nonsterilized grains or seeds inoculated with *A. parasiticus*. Four types of aflatoxins were detected, i.e., B_1, B_2, G_1 and G_2, and the amounts of the unsaturated toxins (B_1 and G_1) were greater than the respective dihydro derivatives (B_2 and G_2). The level of total aflatoxins does not indicate the actual toxicity, because G aflatoxins are about one-half as toxic as their B counterparts. The unsaturated compounds are approximately 4.5 times as potent as their respective dihydro derivatives (18). Therefore, the toxicity index was used to indicate the actual toxicity potential of the mycotoxins calculated in terms of aflatoxin B_1. Sterilized seeds infected with the fungus generally contained greater amounts of aflatoxins than those infected without previous sterilization. Also, visual observation revealed that fungal growth was considerably greater in sterilized substrates than in nonsterilized ones. One could suggest that the sterilization process plays a dual function by causing the breakdown of the outer seed shells and destroying some of the competitive micro-organisms associated with the seed crops. Consequently, these two factors pave the way for growth of *A. parasiticus* and subsequent production of aflatoxins.

Relationship between aflatoxin production and chemical composition of fungal substrate. The chemical composition of the seeds under study was determined (Table 2). The total carbohydrate (C) and lipid:protein (*L:P*) ratio was calculated to deduce the relationship between aflatoxin production and the chemical composition of the seeds. The values of C + L:P ratios of the seeds under study were arranged in the decreasing order; wheat (starchy seeds) > sesame (oily seeds) > peanut (oily seeds) > faba bean (proteiny seeds) > soybean (proteiny seeds). Statistical analyses indicate there was nonsignificant correlation between aflatoxin production and lipid ($r = -0.33$) or carbohydrate ($r = 0.72$).

TABLE 3

Fatty Acid Composition (%) of the Oils Extracted from Wheat, Faba Bean, Soybean, Peanut and Sesame Seeds

Fatty acid	RRT[a]	Wheat	Faba bean	Soybean	Peanut	Sesame
12:0	0.26	0.10	0.80	0.30	0.10	0.10
13:1	0.45	—	0.20	—	0.20	0.10
14:0	0.51	0.30	0.40	0.20	0.10	—
14:1	0.62	—	0.10	—	—	—
15:0	0.74	—	0.10	—	—	—
16:0	1.00	26.10	13.80	20.10	11.20	10.80
17:0	1.43	—	0.10	—	—	—
18:0	2.03	0.90	1.40	3.70	1.50	3.60
18:1	2.23	25.40	41.00	20.70	67.10	53.70
18:2	2.71	45.60	39.50	50.50	19.80	31.70
18:3	3.50	1.50	2.10	4.50	—	—
20:0	3.94	—	0.50	—	—	—

[a]RRT refers to the relative retention time; the retention time of 16.0 was given a value of 1.0.

However, there was a significant correlation between aflatoxin production and C + L:P ratio (r = 0.82 at 5% level). The seeds with high C + L:P ratio produced high amounts of aflatoxins and vice versa. Consequently, C + L:P ratio appears to play an important role in aflatoxin production.

It has been reported that the amount of aflatoxins produced by *A. parasiticus* on various sterilized, non-aged seeds was higher in oily seeds than in starchy seeds (19). In addition, the percentage of polyunsaturated acids (18:2 and 18:3) in the oils was important, probably because the fatty acids are more easily peroxidizable than the monounsaturated fatty acids, and peroxides enhance aflatoxin production.

Table 3 presents the fatty acid composition of the seeds under investigation. Palmitic (16:0), and oleic (18:1) and linoleic (18:2) acids were the most predominant saturated and unsaturated fatty acids, respectively. The present data show no relationship between the amounts of unsaturated fatty acids and aflatoxin production. In fact, wheat kernels (45.5% linoleic and 1.5% linolenic) produced higher amounts of aflatoxin than soybean (50% linoleic and 4.5% of linolenic) or sesame seeds (31.7% linoleic acid). The starchy seeds (wheat) produced the higher amounts of aflatoxins, followed by oily (peanut and sesame) and protein-rich seeds (soybean and faba bean). Consequently, there is a strong relationship between C + L:P ratio and aflatoxin production, but not, as has been proposed previously (19), between polyunsaturated fatty acids and aflatoxin production.

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