Aflatoxin Occurrence in Maize Samples Collected in Haitian Markets

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ABSTRACT

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Sixty-nine percent of 268 samples of corn collected from the retail market in Haiti in January, July, and October 1983 and in January 1984 contained aflatoxin. Twenty-two percent contained greater than 20 ppb (μg/kg) of total aflatoxins, and 10% contained greater than 100 ppb (μg/kg). Corn collected from the city of Gonaives consistently contained the highest incidence and the highest concentrations of aflatoxin. The January harvest appeared to yield more aflatoxin-free maize than the July or October harvests. The incidence of storage fungi found in the kernels was correlated with the incidence of aflatoxin. The most common fungi found in the kernels were *Aspergillus flavus, A. glaucus, A. restrictus, A. ochraceous,* and *Penicillium* species. Inspection of the coarsely ground maize kernels with ultraviolet radiation was an effective way to detect aflatoxin-containing kernels.

Aflatoxins are natural products produced by certain strains of the *Aspergillus flavus* group that are commonly found in the cereal grains. They are important because of their acute toxicity as well as their association with liver cancer in humans (4). Research on the aflatoxins and their effects on animal health has been going on since their discovery in 1960 (1). A wide range of plant seeds and their products grown in both temperate and tropical environments have been reported to contain aflatoxin (2–4,6–9). Among these, maize, cottonseed meal, and peanuts have been found to be high-aflatoxin-risk crops.

In maize, *A. flavus* can colonize the kernels in the field or in storage. The fungus needs a minimum of 18% moisture content on a fresh-weight basis before it can colonize the grain and produce the aflatoxins. Many environmental factors such as storage conditions, irrigation, drought, methods of preparation, and subsequent storage of food will influence the incidence and quantities of aflatoxins produced (10,11).

This study was initiated in Haiti to determine the occurrence of the aflatoxins in the maize crop. We wished to determine the extent of aflatoxin contamination and whether there were any differences in its incidence in different localities and harvest periods.

MATERIALS AND METHODS

Random samples of whole and ground kernels of maize were collected from markets throughout Haiti during January, July, and October 1983 and during January of 1984. The samples ranged from 0.5 to 1 kg and were purchased from venders in a total of 14 marketplaces. Several samples were collected from fields as well as from maize stored outside under the eaves of farm buildings. The samples were placed in cloth bags and transported to the laboratory during the period of 1 wk, then they were placed in a refrigerator at 8 C.

Moisture contents (fresh weight) of maize samples were determined with an electronic moisture meter.

A representative portion (30 seeds) of each sample of whole maize was surface-treated in 1% NaOCl for 1 min and rinsed three times with sterile distilled water. Thirty seeds (10 seeds per dish) were placed on T-6 agar and incubated for 7 days at room temperature (21–24 C). The percentage of field and storage fungi was determined.

The samples were checked for blue-green-yellow (BGY) fluorescence with an ultraviolet lamp emitting at 365 nm. Whole maize samples and ground samples were compared for efficiency in determination of the incidence of aflatoxin contamination.

Analytical determination of the total aflatoxins (B_1, B_2, G_1, and G_2) was done by high-pressure liquid chromatography (5) in the Medallion Laboratories, Minneapolis, MN. Control samples of maize naturally contaminated with aflatoxin and clean samples of maize were used as a measure of the reliability of the method.

RESULTS

Sixty-eight percent of 268 whole and ground maize samples from four independent collections made at different times in Haiti contained detectable quantities of aflatoxin (Table 1). Twenty-two percent contained greater than 20 ppb total aflatoxins, and 28 samples (10%) contained quantities greater than 100 ppb. A good correlation existed between those samples that were positive in the ultraviolet irradiation test (showing characteristic BGY fluorescence) and those that contained aflatoxin. Thus, 76% of the total samples were BGY-positive and 68% actually contained aflatoxin. In the July 1983 collection, all of 100 samples tested were BGY-positive. Ninety-three percent of these contained aflatoxin ranging in concentration between 0.1 and 860 ppb with a mean of 65.1 ppb; 7% were false positive. This means that the BGY test is effective and practical in detecting total aflatoxins; some of the concentrations were less than 20 ppb.

The incidences of aflatoxin-positive samples (35 and 39%, respectively) found in the January 1983 and 1984 collections were significantly lower than those found in the July and October 1983 collections (93 and 94%, respectively).

In January 1984, samples of maize were collected from cities in the northern, central, and southern regions of Haiti to determine whether any incidence or level of contamination differed among regions.

Table 1. Correlation of aflatoxin in maize with blue-green-yellow (BGY) fluorescence

Time of sampling	No. samples	No. samples BGY-positive	Percent samples with aflatoxin total > 20 μg/kg[a]	Aflatoxin (ppb)	
				Mean	Range
Jan. 1983	72	36	35 (6)	10.3	0.1–69
Jul. 1983	100	100	93 (35)	65.1	0.1–860
Oct. 1983	52	32	94 (27)	140.9	0.1–2,858
Jan. 1984	44	36	39 (11)	566.2	0.3–4,501
Total	268	204	68 (22)	124.1	0.1–4,501

[a] Values in parentheses indicate percentage of positive samples that are greater than 20 ppb.

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In the city of Gonaives in the northern region, a higher incidence and quantity of total aflatoxins was found than in other cities (Table 2). Fifty percent of the samples from Gonaives contained aflatoxin ranging in concentration between 6 and 4,501 ppb. The incidence of aflatoxin in Gonaives was high on other sampling occasions as well; i.e., 4 of the 20 highest aflatoxin-containing samples (>100 $\mu g/kg$) contained aflatoxins G_1 and G_2 as well as B_1 and B_2. It is unusual to find all four aflatoxins occurring together; B_1 is found most often. The incidence of aflatoxin B_1 averaged 88% and that of B_2 averaged 12% in the total samples collected. Two percent of the samples contained all four aflatoxins.

The most common fungi identified from the 107 whole kernels of maize were *A. flavus, A. glaucus, A. restrictus, A. ochraceus,* and *Penicillium* species (Table 3). In general, the incidence of *A. flavus* increased as the incidence and concentration of aflatoxin increased. Thus, 32 samples (30%) contained no aflatoxin and a low percentage of *A. flavus*. At the higher concentrations such as those containing a mean of 33, 67, and 925 ppb of aflatoxin, however, the incidence of *A. flavus* was greater. The incidence of other storage fungi, especially *A. glaucus*, was consistently high in all categories.

The relative humidity at several locations in Haiti ranged from 57 to 66% when measured at midday, but early in the morning, it ranged from 71 to 82%. The mean relative humidity was 47% in a desert area south of Gonaives and 46% north of Gonaives near Gros Morne.

A small study was done to determine whether any correlation existed between BGY-positive samples and aflatoxin concentration. The 108 original samples of whole-kernel maize (containing a mean concentration of 182 $\mu g/kg$ of total aflatoxins) were inspected by ultraviolet irradiation as whole kernels. Seventy-two samples were accepted as negative because of a negative BGY test; however, an average of 51 ppb of aflatoxins was found in the 72 samples accepted. The 36 samples rejected because of a positive BGY test contained an average of 446 ppb of aflatoxins. When the 72 samples mentioned were coarsely ground and inspected by ultraviolet irradiation, 52 were BGY-negative and these contained a mean of only 1.1 μg of aflatoxins per kilogram of maize. The other 20 samples, which were BGY-positive, contained a mean of 145 μg of aflatoxin per kilogram maize. It is clear that inspection by irradiation with ultraviolet light is an effective means of detecting aflatoxin in corn provided the corn kernels are coarsely ground.

The mean moisture content of the 58 whole maize samples was 13.4%. The mean moisture contents of maize samples collected in October 1983 and January 1984 were 13.3 and 13.4%, respectively. Only five of the 58 whole maize samples (9%) were above 16.7% moisture content on a wet-weight basis.

Table 2. Sample origin, percentage, and concentration of aflatoxin in maize collected from three regions of Haiti in January 1984

Location	Samples with aflatoxin: No. samples	Samples with aflatoxin: Percent aflatoxin	Concentration (ppb) (ng/g): Mean	Concentration (ppb) (ng/g): Range
Northern				
Gonaives	12	50	1,589	6–4,501
San Marc	4	25	15	15
Central				
Solomon	5	0	0	0
Bouquette	5	20	3	3
La Minoterie	2	100	1.3	1–1.6
Southern				
Les Cayes	14	43	12	0.6–37
Cavaillon	2	50	1.2	1.2
Total	44	39	566	0.3–4,501

Table 3. Aflatoxin concentration and incidence of storage fungi isolated from maize samples collected in July and October 1983 and January 1984 throughout Haiti

Aflatoxin range ($\mu g/kg$)	No. samples	Percent incidence of fungi[a]: AR	AG	AO	AF	AN	P	Aflatoxin mean ($\mu g/kg$)
0	32	8	27	4	1	1	11	0.0
0.1–1	24	6	25	3	4	2	11	0.5
1–5	17	6	27	3	10	11	10	2.7
5–10	3	1	17	0	1	5	9	6.0
10–20	5	15	57	14	7	7	7	14.0
20–50	4	10	27	3	14	22	10	33.6
50–100	2	0	2	0	8	27	0	67.1
>100	20	6	29	7	31	19	6	924.9
Total	107	7	28	5	10	8	9	176.7

[a] AR = *Aspergillus restrictus*, AG = *A. glaucus*, AO = *A. ochraceous*, AF = *A. flavus*, AN = *A. niger*, and P = *Penicillium* spp.

DISCUSSION

Most of the maize in Haiti is grown from April through July; this overlaps one of the two rainy seasons, May through June and October through November. In general, the northern half of the island is drier than the southern. Maize is grown throughout Haiti, however, and when mature in the field, it is allowed to dry by turning down the husk, or if still moist, it is harvested by hand, husked, dried in the sun, shelled, and sold almost immediately. Field-dried corn is harvested with the husks intact and stored primarily as bundles hanging from trees or rafters of homes or on rooftops. (The thick tight husks protect the ears from birds and insects during storage.) The corn is shelled as needed for home consumption. The quantity of maize sold in the market is usually small because most of the farms (about 600,000) are small. Most of the maize in Haiti is consumed by humans rather than by animals.

One of the objectives of this research was to determine if aflatoxin was prevalent in maize sold in the marketplace in Haiti. It was proposed that corn grown in Haiti be used in a government-owned mill for making a high-protein supplement for human consumption called Acamil. A screening program was to be established to detect any aflatoxin-contaminated maize arriving in the mill. No base line existed before this study that indicated the amount of aflatoxin to expect, if any, in homegrown maize. This study was designed to indicate the incidence of aflatoxin in maize after the various harvests for at least 2 yr.

As a result of this survey, we found that the amount of aflatoxin in Haitian corn was considerable; i.e., 22% of 268 samples contained concentrations of aflatoxin greater than 20 ppb. Moreover, 10% of the samples contained total aflatoxins greater than 100 ppb. The latter concentration indicates that careful screening procedures should be established to intercept badly contaminated maize. The above figures are based on maize samples gathered predominantly in the marketplace, where they are sold for human consumption; for the most part, samples were not collected from fields.

We detected a seasonal variation in the incidence of aflatoxin; samples collected in January had a lower concentration of the toxin than those gathered in July or

October. Individual samples, however, collected at any time can have a high concentration of aflatoxin (2,000–4,500 ppb). These can be effectively screened out with the BGY fluorescence technique.

The marketplace in Gonaives, when compared with marketplaces in San Marc or LesCayes, as an example, contained an inordinate amount of aflatoxin. This high concentration was consistent in all collections, but the exact reason for this is not known. We have made the assumption that maize sold in the market originated from the local fields. If this were true, then the maize in the field near Gonaives would be highly contaminated with the toxin. The moisture content of maize sold in the market ranged between 13 and 15% on a wet-weight basis; this argues against the hypothesis that aflatoxin is formed in storage in the market. Much of the maize sold in the market is maize that is already ground, however, and we know very little about the keeping qualities of such corn. Nevertheless, maize originating in Gonaives should be studied in depth to determine the cause of the inordinate amount of aflatoxin.

Storage fungi occurred in abundance in the samples tested. The mean quantity of the low-moisture-requiring *Aspergillus* species (*A. restrictus* and *A. glaucus*) was similar for all aflatoxin groups (Table 3). The *Aspergillus* species requiring higher moisture (*A. ochraceous, A. niger,* and *A. flavus*) increased in occurrence with the increase of aflatoxin in the corn. Several samples were collected from fields or from storage (hanging from eaves) or purchased in the market as newly harvested corn; such samples had very little or no aflatoxin. Thus, contamination appears to arise in storage, although we do not know under what circumstances. Moreover more field samples would have to be collected to formulate a sound conclusion.

The primary method of preparing ground maize for consumption in Haiti is by boiling. Boiling will remove a small portion (20%) but not the major portion of the aflatoxin contamination. Maize tortillas are not used as food in Haiti as they are in other nearby Latin American countries. The use of alkali in manufacture of tortillas reduces a significant amount of the aflatoxin contained in the maize (11).

Use of ultraviolet irradiation for detecting BGY fluorescence is an effective means of detecting lots of maize that contain at least 10 ppb of total aflatoxins. Such screening will be effective in minimizing aflatoxin in maize used in commercial products.

We conclude that maize collected in the marketplace in Haiti contains a sufficient amount of aflatoxin to warrant caution when consumed by humans or animals. The greatest contamination originated in the marketplace in Gonaives. Most of the maize sold in the market is sold as ground maize, and we do not know the storage properties of such maize. Most of the aflatoxin contamination, however, can be detected with ultraviolet radiation of coarsely ground maize by the BGY fluorescence technique.

We recommend that all maize used for human consumption be screened to eliminate any maize with a total aflatoxin concentration greater than 10 ppb. This can be achieved easily using the ultraviolet irradiation for detection. Maize such as this should be safe for incorporation into products used for human consumption. Moreover, the aflatoxin contamination risk can be further reduced by using maize from the fall harvest as represented by the maize available in the marketplace in January.

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