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Aflatoxin Contamination in Grains from the Pacific Coast in Guatemala and the Effect of Storage upon Contamination

Marit de Campos, J. Crespo Santos, and A. E. Olszyna-Marzys
Institute of Nutrition of Central America and Panama (INCAP) Guatemala, C.A.

A monitoring study on aflatoxin contamination in grains and grain products, carried out in Guatemala in 1976, showed a high incidence of contamination (CAMPOS and OLSZYNA–MARZYS 1979). On the southern Pacific coast of Guatemala the temperature and the relative humidity can get very high, conditions that favour aflatoxin contamination. As could be expected, the highest incidence of contamination (26%/o of the samples analyzed) was found in this region (CAMPOS and OLSZYNA–MARZYS 1979). For this reason, it was decided to carry out a more complete study in that same area.

The potential danger involved when mold-infested food is consumed is presently a well-known fact and the toxicity of mycotoxins in general, and especially of the aflatoxins, is well documented. Acute intoxications occur mostly in animals (CAMPBELL and STOLOFF 1974), while chronic intoxications due to the carcinogenic properties of the aflatoxins occur both in man and animals (CAMPBELL and STOLOFF 1974, MILLER 1966, VAN RENSBURG *et al.* 1974).

Because of this, various countries have established maximum limits for aflatoxins in food. In the United States, the FDA applies a 20 ppb (parts per billion) action level for all affected foods, except peanuts where 15 ppb has been proposed (FEDERAL REGISTER 1974).

Guatemala has not yet established any maximum limits for aflatoxins, but in those cases it is usual to employ the FDA regulations or those of the Codex Alimentarius Commission as guidance.

MATERIALS AND METHODS

One hundred and forty-five grain samples were collected from silos, homes and small shops in different villages on the southern coast of Guatemala. The samples were collected in polyethylene bags in quantities between 1 and 2 pounds. The distribution of the samples was as follows:

Part I. Forty-five samples of corn, coffee, beans, sorghum, rice and sesame seeds, stored for six months during the dry season, were collected. It is common practice in the area to fumigate grain that is to be stored over long periods of time with CS_2 (carbon disulfide) for protection against insects. The corn and sorghum samples collected for the study had been treated with CS_2.

Part II. Fifty duplicate samples of corn, beans and rice were collected within 20 days after harvest, which took place in June, in the middle of the rainy season. As corn is the most common staple food in Guatemala, most of the collected sam-

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TABLE I

Aflatoxin Contamination in Grains Stored for Six Months During the Dry Season in Guatemala, C. A. (in ppb)

Commodity	Samples analized n	Positive samples n	Contaminated samples n				Maximum value	Aflatoxins present (in 1 sample or more)
			<5	6–20	21–100	>100		
Corn*	18	3			3		30	$B_1+B_2+G_1+G_2$
Sorghum*	9	3	1	1	1		25	B_1+B_2
Sesame seed	6	1	1				5	B_1
Black beans	4	4		4			20	$B_1+B_2+G_1$
Coffee	4	2			2		37	B_1
Rice	4	1			1		30	B_1+B_2
Total	45	14	2	5	7	0		
Positive samples, percent of total		31%	4%	11%	16%			

* Treated with CS_2

ples were corn samples (42 in 50 samples). One set of duplicates was immediately taken to the laboratory for analysis and the other set was stored for 2 months under local conditions, on shelves under the kitchen roof. These samples were not treated in any way, as this is not usual when the storage period is short. During the storage period the temperature ranged from 20 to 37°C and the relative humidity from 62 to 95°/o.

Once received in the laboratory, the samples were ground and moisture determinations were made immediately. When the aflatoxin analysis could not be carried out at once, the samples were stored at −20°C to inhibit further mold growth.

All the samples were analyzed by the ROMER Method (1975) with the modifications described by CAMPOS and OLSZYNA–MARZYS (1979). Uncontaminated samples fortified with 20 ppb of aflatoxin B_1 gave a mean recovery of 82°/o. The mean coefficient of variation between duplicates was 9°/o. A "Velasco Flurotoxin Meter" (Neotec Instruments, Inc.) was used for screening and the presumptive positives were confirmed by TLC (thin layer chromatography). Basically, the AOAC Method (1975) was employed, but benzene-acetic acid (9+1) was used as developing solvent with very good results (VEGA 1976). Heavily contaminated samples were also confirmed by HPLC (high pressure liquid chromatography), using the conditions reported by PONS and FRANZ (1978). Twenty microliters of the first chloroform extract from the ROMER Method was injected directly onto the HPLC. It is important to confirm the positive samples by other methods. In our case, about one third turned out to be false positives, meaning that the fluorescence was due to substances other than aflatoxins.

RESULTS AND DISCUSSION

As can be seen in Table I, a very high proportion (31°/o) of the samples stored for 6 months during the dry season were contaminated with aflatoxins, 16°/o exceeding the 20 ppb level. The maximum value of 37 ppb was found in a coffee sample.

The results of Part II of the study are reported in Table II. In the first set of the 50 duplicate samples, harvested during the rainy season and collected for analysis within 20 days after harvest, 16°/o were found positive; 6°/o exceeded the 20 ppb level with a maximum value of 130 ppb.

The same samples analyzed 2 months later showed a high increase in aflatoxin contamination: 24°/o were contaminated, 16°/o had values higher than 20 ppb and 12°/o higher than 100 ppb. The maximum value, 1,650 ppb, was found in corn. None of the three rice samples were contaminated.

No correlation could be found between moisture content and aflatoxin contamination. However, the moisture content of most of the samples stored for 6 months was relatively low, the mean ± standard deviation ($\bar{x} \pm$ S.D.) was 12.7 ± 3.2°/o, which is considered close to the critical humidity level for aflatoxin formation. The rest of the samples had a high moisture content 20 days after harvest; $\bar{x} \pm$ S.D. was 17.1 ± 2.8°/o while after 2 months of storage the value was 13.6 ± 1.3°/o. The critical humidity level varies from product to product (FAO/OMS/PNUMA 1977, ZINTZEN 1975), but a moisture content higher than 13°/o is considered critical for many products. For corn and corn products the humidity

TABLE II

Aflatoxin Contamination in Grains Harvested and Stored During the Rainy Season
(in ppb)

Commodity	Samples analyzed n	Positive samples n	Contaminated samples n				Maximum value	Aflatoxins present (in 1 sample or more)
			<5	6–20	21–100	>100		
Corn	42	(7)*10**	(0) 3	(4) 0	(2) 1	(1) 6	(130) 1650	$B_1+B_2+G_1+G_2$
Black beans	5	(1) 2		(1) 1	(0) 1		(10) 30	$B_1+B_2+G_1+G_2$
Rice	3	(0) 0						
Total	50	(8) 12	(0) 3	(5) 1	(2) 2	(1) 6		
Positive samples, percent of total		(16) 24%	(0) 6%	(1 0) 2%	(4) 4%	(2) 12%		

* In parenthesis: Analyzed within 20 days after harvest.

** Outside parenthesis: Same samples after 2 months of storage.

should be less than 13°/o for storage up to 1 year; for longer periods, 11°/o is recommended (ZINTZEN 1975).

In Table III the visible insect damage and the relation between insect damage and aflatoxin contamination of the samples is indicated. As can be seen, the visible insect damage was relatively low for the samples of Part I, stored for 6 months during the dry season. The samples of beans, rice, coffee and sesame seeds were not damaged at all, while for corn and sorghum, both treated with CS_2, the damaged samples represented 7°/o. Of the contaminated samples only 7°/o were damaged by insects.

For the samples of Part II, collected during the rainy season, the situation was quite different. Insect damage was found in 32°/o of the samples analyzed within 20 days after harvest and in 70°/o of the same samples analyzed after 2 months of storage. When counting only the contaminated samples, a very high proportion had been attacked by insects. Even in the samples collected within only 20 days after harvest, 75°/o were damaged, and after 2 months, as much as 83°/o.

The great difference between Part I and Part II indicated that even if 31°/o in Part I were contaminated with aflatoxins, the danger of finding very high values is much less during the dry season. The results also seem to indicate that the treatment against insects lowered the aflatoxin contamination. This confirms the results of various investigators who claim a good correlation between insect damage and mold invasion with subsequent aflatoxin contamination (FAO/OMS/PNUMA 1977, LILLEHOJ and HESSELTINE 1977). It is not so certain, however, that CS_2 in uncontrolled quantities as is usually employed in the area, is the best solution. It is known that the inhalation of its vapors, whose rate of decomposition is not known, may cause various symptoms, especially in the central nervous system (VETTORAZZI 1976). The FDA permits CS_2 for fumigation of grain-mill machinery and corn grits and cracked rice for fermented malt beverages, but no tolerances exist for residues (U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE 1974).

CONCLUSIONS AND RECOMMENDATIONS

Grains from the hot and humid southern coast of Guatemala are very susceptible to aflatoxin contamination, especially during the rainy season. It is considered that corn represents a special problem: it is the most important staple food in the country and in corn both a high incidence and high levels were found.

About 80°/o of the contaminated samples collected during the rainy season were damaged by insects, indicating the need to use some form of grain protection in order to inhibit mold growth and aflatoxin contamination.

A definite rise in incidence and levels of contamination was found during storage under the prevailing hot and humid conditions. This emphasizes the need for educational programs on the subject. People in general know little about the aflatoxin problem and should be informed of its potential hazard and the way to diminish the contamination.

Studies on storage conditions should be carried out in the field under the prevailing climatic conditions. The habits of the native population must be taken into account in order to ensure that advice and recommendations will be followed.

TABLE III

Insect Damage and Aflatoxin Contamination

Commodity	Damaged samples/Total analyzed			Damaged samples/Contaminated samples		
	Part*	Part II**		Part I	Part II	
		1	2		1	2
Corn	2/18	13/42	35/42	1/3	5/7	8/10
Black beans	0/4	3/5	2/5	0/4	1/1	2/2
Rice	0/4	0/3	0/3	0/1	0/0	0/0
Coffee	0/4			0/2		
Sorghum	1/9			0/3		
Sesame seeds	0/6			0/1		
Total	3/45	16/50	35/50	1/14	6/8	10/12
Percent	7%	32%	70%	7%	75%	83%

* Part I: Samples stored for 6 months during dry season

** Part II 1: Samples analyzed within 20 days after harvest

Part II 2: Same samples, stored for 2 months during rainy season

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