Determination of Aflatoxin and Aflatoxin-Producing Cultures in Recently Ginned Cottonseed in Central America

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ABSTRACT

Aflatoxins were found in four of the 99 samples of recently ginned cottonseed analyzed. From these same 99 samples, four cultures of *Aspergillus* and one of *Penicillium* capable of producing aflatoxin were isolated. In only one case was an aflatoxin producer isolated from an aflatoxin contaminated sample.

INTRODUCTION

For over a decade the presence of aflatoxin has been studied in various agricultural commodities (1). Concern developed in the Central American area as a result of a series of annual meetings at the Instituto Centroamericano de Investigación y Tecnología Industrial with industrial representatives from throughout the region (2,3). Cotton is one of the main export crops for the region, and FAO estimates that 285,000 metric tons of cottonseed were produced in the area in 1970 (4).

Whitten (5) studied the incidence of aflatoxin in cottonseed in the U.S. during the 1964-65 season, and found that 4% of the samples contained over 30 ppb of aflatoxin B_1.

The objective of the present work was to evaluate the level of contamination in freshly ginned seed and the potential contamination due to the presence of aflatoxin producing strains of *Aspergillus* and *Penicillium*.

EXPERIMENTAL PROCEDURES

Sample Collection

Ninety-nine samples of freshly ginned cottonseed were collected during the harvest season (November to April 1971) at various gins throughout the cotton-producing areas of Guatemala, El Salvador and Nicaragua. The cottonseed was maintained in sealed polyethylene bags until it could be analyzed at the Institute. The bags were maintained at room temperature, 22-25 C. The moisture content of the seeds at time of sampling varied from 8 to 17%.

TABLE I

Distribution of Samples Analyzed

Month	Guatemala	El Salvador	Nicaragua
November 1970	7	5	—
December 1970	36	—	2
January 1971	9	—	10
February 1971	12	4	5
March 1971	—	4	—
April 1971	5	—	—
Total	69	13	17

TABLE II

Mold Count Per Gram of Cottonseed

Month	Guatemala	El Salvador	Nicaragua
November	430,000	40,000	—[a]
December	34,000	—[a]	4000
January	10,000	—[a]	2800
February	7400	54,000	4400
March	—[a]	27,000	—[a]
April	4800	—[a]	—[a]

[a]Samples were not analyzed from these countries during this month.

Mold Count

Five grams of each sample was blended with 100 ml Butterfield buffer solution for 15 sec in a Waring blendor. The buffer solution also contained 100,000 IU penicillin and 0.1 g streptomycin to inhibit bacterial growth. After 1 hr of rest, nonacidified potato dextrose agar was inoculated with the adequate dilutions of the above suspension. The agar also contained 10,000 IU penicillin and 0.01 g streptomycin per 100 ml. The plates were incubated at room temperature for 3-5 days before counting.

Isolation of Aflatoxin-Producing Cultures

In order to isolate cultures of *Aspergillus* and *Penicillium*, the procedure described above was followed, blending the seeds in the buffer solution for 5 min instead of 15 sec. After 5 days of incubation at room temperature, characteristic colonies were picked and maintained on potato dextrose agar slants, with subsequent identification by microscopic observation.

Aflatoxin Production

The identified cultures were then tested for their ability to produce aflatoxin on cottonseed meal according to the method used by Mayne et al. (6). Presence of aflatoxin was determined by the SRRL fast screening method (7). This method allows the technician to perform an entire analysis in ca. 20 min. A faint fluorescence appears on the silica column at levels of 10-20 μg/kg. When a sample was considered positive, confirmation was obtained by the method of Pons (8).

Analysis of the Cottonseed for Aflatoxin

The Pons method was used to determine the presence of aflatoxin in the 99 samples (8). The developing solvents mixture was modified to the following proportions by volume:ether-methanol-water 96:3:1. Confirmation of the presence of aflatoxin was obtained by spraying the developed thin layer chromatographic plate with a 50% solution of sulphuric acid and observing the change in fluorescence color to yellow-green.

RESULTS AND DISCUSSION

Table I illustrates the distribution by month of the samples of cottonseed analyzed during this study.

Table II presents the logarithmic mean of the mold counts obtained each month during the harvest season. The

TABLE III

Aflatoxin in Cottonseed

Sample no.	Country	Month	Aflatoxin, μg/kg B_1	B_2	G_1
3	El Salvador	November	50	15	
79	Nicaragua	January	30		
95	El Salvador	March	30	9	5
97	Guatemala	April	60		

mean of the logarithms of the counts was used in order to obtain a more representative value. The counts from the Nicaraguan and Salvadorian samples were relatively constant, although the counts from El Salvador are 10 times greater than those of Nicaragua. The samples from Guatemala decrease considerably throughout the season. We assume that this is due to changes in climatic conditions in the area: in Guatemala the rainy season lasts into November with occasional showers and a generally humid atmosphere. In December and January the weather becomes drier, and the temperature rises until the end of April. In El Salvador and Nicaragua, the harvest lasts for a shorter period which falls entirely within the dry season. In the cotton-producing areas during the dry season, the average monthly rainfall is ca. 5 mm, and the mean temperature is 27 C.

Fifty-two cultures of *Aspergillus* and *Penicillium* were isolated from 44 of the 99 samples. Of these 52, only four cultures of *Aspergillus* and one of *Penicillium* were able to produce aflatoxin on the cottonseed meal medium. It is important to note that these cultures represent the analysis of 495 g cottonseed. Had larger quantities been analyzed, there is no doubt that more aflatoxin producers could have been found. The distribution of the aflatoxin-producing cultures is as follows: Guatemala, one in November and two in December; El Salvador, one in November and one in March; Nicaragua, none.

Aflatoxin was found in four samples (Table III). The 4% incidence is the same as that reported by Whitten (5).

Interestingly, from only one of the aflatoxin contaminated samples, number 95, was isolated an aflatoxin-producing culture. Probably none was found in the other samples due to the absence of spores of the indicated type in the 5 g sample. Also, sample 95 was the only one which contained aflatoxin G. The natural occurrence of aflatoxin G in cottonseed is rather unusual; nevertheless Mayne et al. (6) cite the presence of aflatoxin G in a sample of Sudanese cottonseed cake.

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