

Performance of visual and chemical methods in identifying aflatoxin contamination in farmers stock peanuts(1)

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Abstract. — Loads of florunner farmers stock (FS) peanuts were used to evaluate two methods to determine aflatoxin contamination. The two methods evaluated were the visual inspection of kernels for the presence of *Aspergillus flavus* (VAF) and an enzyme-linked immunosorbent assay (ELISA). In loads with greater than 20 ppb aflatoxin, the percentage of samples identified as contaminated was 59.3% and 70% for the VAF and ELISA methods, respectively. Thus, the ELISA tests should help identify loads of peanuts contaminated with aflatoxin, resulting in higher quality peanuts reaching consumers.

Key words. — Groundnut, aflatoxin, *Aspergillus flavus*, immunoenzymes, toxicology

INTRODUCTION

Aflatoxin is a toxic material that can be produced in peanuts (*Arachis hypogaea* L.) by the fungi *Aspergillus flavus* and *A. parasiticus* (hereafter collectively termed *A. flavus*). During the grading of farmers stock (FS) peanuts in the U.S., the loose shelled kernels from a 1.8 kg sample and the kernels that are shelled from 500 g subsample of pods are visually examined for the presence of these fungi (VAF method). If *A. flavus* is found, the load of peanuts is presumed to be unacceptably contaminated with aflatoxin, the load is classified as Segregation 3, and it is diverted to oil stock. However, classification errors may occur for any one of the following reasons: the sample may not represent load, the inspector may misclassify harmless fungi as *A. flavus*, the *A. flavus* may be correctly identified but may not have produced aflatoxin, and aflatoxin may be present in the peanuts but the *A. flavus* may not have been identified by the inspector. If aflatoxin is present but *A. flavus* is not identified, then contaminated loads of peanuts will be mixed with loads that are aflatoxin-free. On the other hand, if the inspector correctly or incorrectly identifies a load as having *A. flavus* but no aflatoxin present, the edible peanuts will be diverted to oil stock.

Sample size errors will occur with any testing procedure; however, the subjectivity and indirectness inherent to the VAF method can be eliminated through the use of a chemical aflatoxin test. Because of the low tolerances for aflatoxin (20 ppb in the U.S. and 10 ppb in some peanut-importing countries) in edible peanuts, it is vital that the best possible

aflatoxin determination be made on FS peanuts. Therefore, it is important to study, evaluate, and recommend needed changes and/or improvements in the current aflatoxin control program for FS peanuts.

Although analytical methods for aflatoxin determinations, including thin-layer chromatography (TLC), minicolumn chromatography, and high-performance liquid chromatography (HPLC), have been available for some time, an analytical method for FS peanuts has not been adopted (Davidson *et al.*, 1984; Holaday, 1976). Reasons for this include the cost and time necessary for analysis. Recently, several enzyme-linked immunosorbent assays (ELISA) have been introduced (Dorner and Cole, 1989; Pestka *et al.*, 1980; Koeltzow and Tanner, 1989). Many of these are rapid and relatively inexpensive methods that may make feasible and practical a direct analysis of FS peanuts for aflatoxin.

The objective of this study was to compare the performance of the VAF and ELISA methods in identifying contaminated loads of FS peanuts.

MATERIALS AND METHODS

In the 1986 crop year, the accuracy and variability in sampling and grading florunner FS peanuts were studied. During this experiment, the VAF and ELISA methods were used to collect data for improving the identification of aflatoxin contamination in FS peanuts. The schematic procedures of the study is shown in Fig. 1. After marketing, separate Federal State Inspection Service approved pneumatic sampling patterns were used to take each of 3, 20 kg samples. The trailer load was then unloaded and sampled with an automated spout sampler to take one 60 kg sample. The 60 kg sample was divided into 3, 20 kg subsamples by use of the official farmers stock divider. After sampling, the peanuts from each trailer were weighed and shelled at a shelling plant to determine shelling outturns and grade factors on the basis of the total load.

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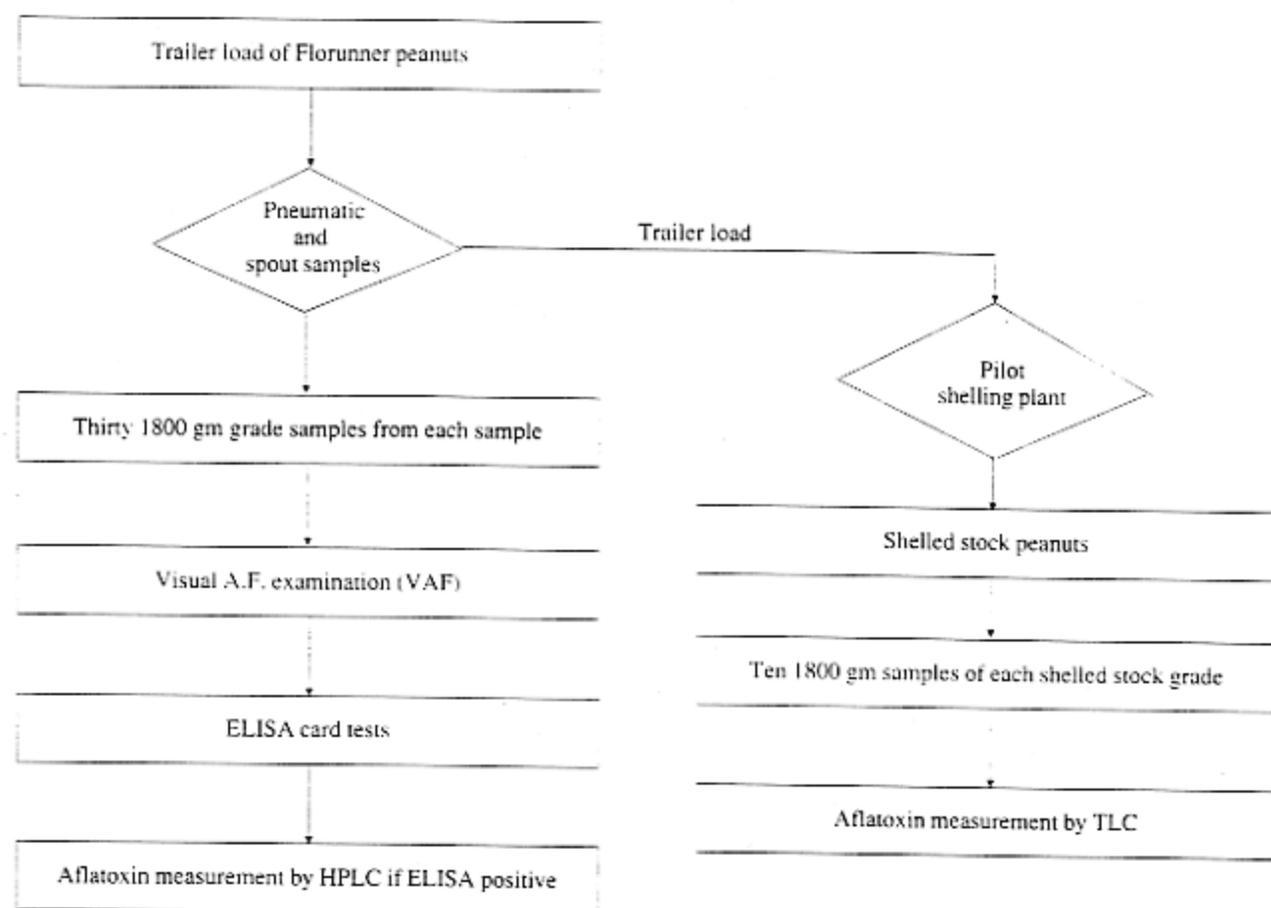


FIG. 1. — Schematic procedures of the experiment to compare aflatoxin detecting methods and to measure aflatoxin contamination

□ Shelling plant methods

The shelled stock was segregated into edible kernels consisting of whole kernels riding a 16/64 inch slotted screen and split kernels riding a 17/64 round hole screen and inedible kernels which consisted of small whole and split kernels falling through these screens and all loose shelled kernels. Ten samples (approximately 1.8 kg each) were taken from the edible and inedible categories of each load for determination of aflatoxin concentrations.

Measurement of aflatoxin in samples of shelled stock peanuts was performed by the Fruit and Vegetable Processing and Products Division, USDA, AMS, Albany, GA, and analyzed by TLC. As indicated by results of ELISA tests on grade samples, 4 out of 13 trailers (1, 2, 3 and 6) were not suspected of containing aflatoxin. Thus, the edible stock from these 4 loads were not evaluated by the TLC method.

□ Pneumatic and spout sample methods

Each of the 20 kg FS peanut samples and subsamples obtained from the pneumatic and spout samplers was subdivided into 10 samples of 1.8 kg each. Thus, there were 60, 1.8 kg samples per trailer (3 pneumatic samples + 3 spout subsamples × 10 samples). From each 1.8 kg sample, the LSK and the kernels from a 500 gram sample of pods were inspected for visible *A. flavus*. Then the same LSK and kernels were ground and subsampled for the analytical tests. Each subsample was analyzed for aflatoxin with the EZ-Screen Quick Card test (Environmental Diagnostics, Inc., Burlington, NC 27215) to indicate either a positive (20 ppb)

or negative (ppb) result. If there was a positive result by the ELISA test, the same extract was subsequently analyzed by HPLC (Dorner and Cole, 1988) to determine the level of contamination. Dorner and Cole (1989) showed that the ELISA test identified 20 ppb samples 99% of the time. Negative ELISA samples were not analyzed by HPLC in order to keep the number of analyses manageable.

RESULTS AND DISCUSSION

The aflatoxin levels indicated by the TLC method in each of 13 loads of florunner peanuts are presented in table 1. Weighted means of aflatoxin in the total grade ranged from 0.1 to 179.3 ppb. These results indicated that all of the 13 trailers were contaminated. There were 5 loads that had total aflatoxin contamination greater than 5 ppb. There were only 4 loads that had more than 5 ppb aflatoxin in the edible stock.

As expected, aflatoxin contamination in the edible stock was generally less than those in the inedible stock. The difference in contamination between the edible and inedible stock was proportional to the level of contamination. Table 1 shows that the range of aflatoxin means for each trailer was generally greater in the inedible than in the edible. The range increased as the weighted mean of aflatoxin in the load increased.

A trailer by trailer comparison of the VAF and ELISA methods is shown in table II. Since all of the 13 trailers were contaminated (Table 1), the best method of detection should

TABLE I. — Aflatoxin levels (ppb) as determined by the TLC method in grade samples from 13 loads of farmers stock peanuts

Trailer	Aflatoxin weighted mean			Range of shelled grade sample means			
	Total	Edible	Inedible	Edible		Inedible	
1	0.1	nd ⁽¹⁾	0.5	nd	0.0	-	1.4
2	0.6	nd	3.3	nd	0.0	-	9.0
3	0.9	nd	3.5	nd	0.0	-	4.8
4	1.3	1.3	0.9	0.0	-	2.9	0.4
5	2.0	0.2	15.6	0.0	-	0.8	3.7
6	2.6	nd	15.1	nd	-	-	0.0
7	3.1	0.3	11.3	0.0	-	1.3	2.2
8	4.1	0.0	31.9	0.0	-	0.0	0.0
9	20.6	21.8	16.5	0.0	-	47.8	1.0
10	49.3	0.3	428.0	0.0	-	0.8	51.2
11	52.2	18.6	169.5	0.0	-	59.0	81.7
12	106.8	86.8	150.7	6.8	-	154.0	138.0
13	179.3	121.5	594.4	6.0	-	298.0	138.9

(1) Aflatoxin not detected by the ELISA test and thus not analyzed using TLC

TABLE II. — Performance of VAF and ELISA methods in identifying aflatoxin-contaminated loads using grade samples from loads of farmers stock peanuts

Trailer	Total ppb	No of samples	% of samples with $\geq A. flavus$ kernels by VAF method	% of samples with ≥ 20 ppb by ELISA method
1	0.1	45	0.0	15.6
2	0.6	60	0.0	8.3
3	0.9	59	0.0	8.5
4	1.3	60	1.7	6.7
5	2.0	59	0.0	5.1
6	2.6	57	0.0	8.8
7	3.1	60	3.3	16.7
8	4.1	60	0.0	3.0
9	20.6	51	47.0	64.7
10	49.3	60	0.0	3.0
11	52.2	53	88.7	92.4
12	106.8	58	69.0	91.4
13	179.3	60	91.7	96.7

TABLE III. — Performance of the ELISA method using the HPLC method as a check

Trailer	Total ppb	ELISA samples that agreed with HPLC	Total samples ⁽¹⁾ analyzed by ELISA	% ELISA that agreed with HPLC	HPLC mean when ELISA agreed (ppb)	HPLC mean when ELISA disagreed (ppb)
1	0.1	8	14	57.1	4.8	0.3
2	0.6	14	19	73.7	1.7	0.6
3	0.9	13	17	76.5	14.8	0.8
4	1.3	33	36	91.7	2.2	0.3
5	2.0	39	44	88.6	35.5	5.4
6	2.6	8	11	72.8	1.5	0.0
7	2.6	27	38	71.0	58.8	2.0
8	3.1	9	12	75.0	0.1	0.3
9	4.1	40	44	90.9	282.2	4.2
10	20.6	22	29	75.9	0.0	4.7
11	49.3	50	51	98.0	634.0	1.0
12	52.2	53	56	94.6	318.9	12.7
13	106.8	58	60	96.7	965.2	48.5
	179.3					

(1) Total samples are less than 60 because negative ELISA samples were not analyzed by HPLC

identify a higher percentage of samples as positive regardless of the range of aflatoxin concentration. For the 5 loads having significant aflatoxin (20 ppb) in the total load, the percent of sample identified as being contaminated averaged 59.3% and 70.0% for the VAF and ELISA methods, respectively. For the other 8 loads that had lower levels of aflatoxin, the percent of samples identified averaged 0.6% and 9.3% for the VAF and ELISA methods respectively. Thus, the ELISA test identified a higher percentage of lots with ≥ 20 ppb aflatoxin than the VAF. This is important if increasing quality and reducing the number of contaminated loads that reach the consumer is important. The ELISA method did reject more ppb contaminated loads; therefore, the econo-

mics of this higher rejection rate would have to be weighed against the quality increase if the peanut industry were to adopt the ELISA testing procedure.

A comparison of the ELISA method with the HPLC method is presented in table III. The agreement of the ELISA positive test with HPLC was good (90%) in the 5 loads having ≥ 20 ppb total aflatoxin. In the other 8 loads that had low levels of aflatoxin, the ELISA and HPLC tests agreed 75.8% of the time. Of the tests that agreed from the 8 loads with ≥ 20 ppb aflatoxin, the HPLC mean of aflatoxin was 14.9 ppb. Furthermore, the aflatoxin means at which the ELISA method did not agree with the HPLC method were low (with HPLC subsamples mean of 1.2 ppb), as compared to the cri-

tical concentration of 20 ppb at which the ELISA cards were manufactured. The ELISA method rejected more loads than the chemical methods when compared to research by Davidson *et al.* (1984) and Whitaker (1986). This may be due to the differences in the chemical test being used or to the distribution of aflatoxin in the samples attributable to the environmental conditions of that crop year.

In summary, the results demonstrated that the ELISA method was more sensitive than the VAF method in identifying

aflatoxin contamination in FS peanut loads. Therefore, new procedures for testing FS peanut loads for aflatoxin warrants consideration as a step towards achieving a satisfactory aflatoxin control program.

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RESUME

Performance des méthodes visuelles et chimiques utilisées pour l'identification de la contamination par aflatoxine de stocks d'arachide chez les planteurs

F.E. DOWELL, Y.J. TSAI, J.W. DORNER, R.J. COLE et J.I. DAVIDSON, JR. *Oléagineux*, 1992, **47**, N° 10, p. 583-586

Des lots d'arachide de type florunner provenant des stocks de planteurs ont été utilisés pour évaluer deux méthodes employées dans la détermination de la contamination par aflatoxine. Il s'agit de l'inspection visuelle des graines afin de détecter la présence d'*Aspergillus flavus* (VAF) et d'un test immunoenzymatique (ELISA). Dans des lots présentant une teneur en aflatoxine supérieure à 20 ppb, le pourcentage d'échantillons identifiés comme étant contaminés s'élevait respectivement à 59,3 % pour la méthode VAF et à 70 % pour la méthode ELISA. Les test ELISA devraient donc contribuer à identifier les lots d'arachides contaminés par aflatoxine, assurant ainsi une meilleure qualité des arachides livrées aux consommateurs.

Mots clés. — Arachide, aflatoxine, *Aspergillus flavus*, immunoenzymes, toxicologie.

RESUMEN

Resultados de métodos visuales y químicos empleados para identificar la contaminación por aflatoxina de existencias de maní junto a los cultivadores

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Partidas de maní de tipo florunner procedentes de existencias de cultivadores, se emplearon para evaluar dos métodos usados en la determinación de la contaminación por aflatoxina. Se trata de la inspección visual de las semillas, a fin de descubrir la presencia de *Aspergillus flavus* (VAF) y de prueba inmunoenzimática (ELISA). En partidas con contenido de aflatoxina mayor de 20 ppb, el porcentaje respectivo de muestras identificadas como contaminadas era de un 59,3 % para el método VAF y de un 70 % para el método ELISA. Así que la prueba de ELISA habría de contribuir en identificar las partidas de maní contaminadas por aflatoxina, proporcionando así una mejor calidad de los manís entregados a los consumidores.

Palabras claves. — Maní, aflatoxina, *Aspergillus flavus*, inmunoenzimas, toxicología