DETECTING FARMERS' STOCK PEANUTS CONTAINING AFLATOXIN BY EXAMINATION FOR VISIBLE GROWTH OF ASPERGILLUS FLAVUS

by

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One ton of farmers' stock peanuts containing large amounts of aflatoxin may contaminate several tons of good peanuts if mixed with them in storage. Because present methods for aflatoxin assay are time-consuming and costly, analysis of farmers' stock peanuts during marketing is not practicable. Consequently, contaminated peanuts might be inadvertently mixed with clean peanuts. Such mixing could cause the processor considerable expense to remove the contaminated kernels so that the remainder would be acceptable for edible purposes. Sometimes all of the contaminated kernels cannot be removed and a large loss results.

Because members of the *Aspergillus flavus* group of fungi are generally regarded as the primary producers of aflatoxin in peanuts (1,2), peanuts containing kernels with visible growth of these fungi may be suspected to contain aflatoxin. If present, *A. flavus* LINK would probably be visible on damaged kernels in grade samples. Although positive identification of fungi is meticulous and time-consuming, *A. flavus* has certain visible characteristics, described by RAPER & FENNELL (4), that differentiate it from many of the other molds that grow on peanuts.

This paper reports the results of a study of the relationship between aflatoxin contamination in farmers' stock peanuts and the presence of visible *A. flavus* on other kernels and damaged kernels in the official grade samples taken from the peanuts.

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PROCEDURE

The Federal-State Inspection Service helped collect 2,107 grade samples at random from farmers' stock Virginia, Spanish, and runner-type peanuts marketed in Georgia during the fall of 1966. Sound mature kernels, other kernels, and damaged kernels were separated by licensed inspectors according to the official grading procedure, and the other kernels and damaged kernels from each sample were examined for visible *A. flavus*.

Examinations were made by persons who were initially neither formally trained nor experienced in the identification of fungi. The examiners were trained by showing them kernels with visible *A. flavus* and by teaching them to recognize certain morphological characteristics. Instruction lasted less than 1 hour and was followed by intermittent close supervision for 2 to 3 hours. A stereomicroscope with 25× magnification was used for close examination of suspect kernels to determine if the color of the fungus was yellow-green, olive-brown or brown and to observe the shape of the conidial heads. On the average, approximately 1/2 minute was required to inspect a sample. More time, however, was required for samples with a large percentage of damaged kernels or when suspect kernels were examined microscopically.

After visual examination, the samples were analyzed for aflatoxin using the method of PONS & GOLDBLATT (4). All of the damaged and sound mature kernels in each sample were ground together and subsampled for aflatoxin analysis. Since in the milling operation small shriveled kernels can be separated easily by screening, these kernels were not included in the aflatoxin analysis despite the fact that they often contained the only visible *A. flavus*.

Small portions of 152 kernels with tentatively identified *A. flavus* were cultured to check the accuracy of the visual identification. The pieces of kernel were surface disinfected in 1 % sodium hypochlorite for 1 minute, rinsed in sterile distilled water, and incubated on Czapek's Dox (Difco) agar with 3 % sucrose. The cultured pieces were examined after 4 days and at 2-day intervals for 10 days. The fungi growing from the pieces of kernel were either identified in the culture dishes or subcultured for later identification.

RESULTS

Although the weather in Georgia was favorable for peanut harvesting during the harvesting season of 1966, approximately 5 % of 2,107 samples collected from farmers' stock peanuts contained aflatoxin (Table I). (i) For peanuts with 0.0–2.5 % damage, 46 of 84 samples with tentatively identified *A. flavus* contained aflatoxin, averaging 230 μg of aflatoxin per kg of peanuts or an average of 125 μg/kg for all 84 samples. Twenty-three of 1,704 samples with no detected *A. flavus* contained an average of 41 μg/kg or an average

TABLE I

*Relationship of aflatoxin contamination to visible Aspergillus flavus growth on damaged kernels and other kernels in samples of farmers' stock peanuts.**)

Percent damage	No. of samples inspected	Samples with visible A. flavus				Samples with no visible A. flavus			
		No. detected	No. contaminated w/aflatoxin	Avg. µg/kg aflatoxin in cont. samples	Avg. µg/kg aflatoxin in all samples	No. observed	No. contaminated w/aflatoxin	Avg. µg/kg aflatoxin in cont. samples	Avg. µg/kg aflatoxin in all samples
0—2.5	1788	84	46	230	125	1,704	23	41	0.5
2.5—5.5	256	14	10	1,439	1,028	242	20	35	3.0
Over 5.5	63	6	5	2,951	2,459	57	4	70	5.0

*) Amounts of aflatoxin B_1 are shown in the table.

of 0.5 μg/kg for all 1,704 samples. (ii) For peanuts with 2.5–5.5 % damage, 10 of 14 samples with tentatively identified *A. flavus* contained aflatoxin averaging 1,439 μg/kg, or an average of 1,028 μg/kg for all 14 samples. Twenty of 242 samples with no detected *A. flavus* contained an average of 35 μg/kg or an average of 3 μg/kg for the 242 samples. (iii) When damage in the peanuts exceeded 5.5 %, 5 of 6 samples with tentatively identified *A. flavus* contained aflatoxin averaging 2,951 μg/kg, or an average of 2,459 μg/kg for all 6 samples. Four of 57 samples with no detected *A. flavus* contained an average of 70 μg/kg, or an average of 5 μg/kg for all 57 samples.

TABLE II

*Fungi yielded from cultures of 152 peanut kernels having visible fungal growth tentatively identified as Aspergillus flavus by microscopic examination.**)*

Fungus Yielded	No. Kernels Yielding Fungus		Fungus Yielded	No. Kernels Yielding Fungus	
	Mixed Culture	Pure Culture		Mixed Culture	Pure Culture
Aspergillus flavus	24	108	*Aspergillus niger*	2	0
Aspergillus versicolor	1	1	*Rhizopus*	22	4
Aspergillus amstelodami	0	3	*Fusarium*	1	2
Aspergillus ruber	0	2	*Rhizoctonia*	0	1
Aspergillus tamarii	1	1	*Unknown*	0	3

*) A stereomicroscope with 25× magnification was used.

When pieces of kernel with tentatively identified *A. flavus* were cultured, *A. flavus* grew from 132 of 152 cultured pieces (87 %) (Table II). One hundred and eight pieces yielded pure cultures of *A. flavus* and 24 yielded *A. flavus* mixed with other fungi, mostly *Rhizopus*. Several other fungi grew from the pieces, including 5 other species of *Aspergillus*.

DISCUSSION

Tentative identification of *A. flavus* on peanut kernels by visual examination was shown to be correct 87 % of the time by subsequent cultures of the kernels. From 1788 samples with 0.0–2.5 % damage, 46 of 84 samples tentatively identified to have visible *A. flavus* contained an average of 230 μg of aflatoxin per kg of peanuts compared to an average of 41 μg/kg in 23 of 1,704 samples with no *A. flavus* detected. Among 319 samples with more than 2.5 % damage, 15 of the 20 samples tentatively identified to have visible *A. flavus* contained aflatoxin and averaged 1,676 μg/kg compared

to an average of 41 μg/kg in 24 of 298 samples with no *A. flavus* detected.

The fact that many of the samples with kernels classified as containing *A. flavus* were found not to contain aflatoxin may result from one or more of the following:

(i) Fungal growth on kernels in some of the samples (perhaps 13 % or more) was incorrectly identified by the examiners as *A. flavus*.

(ii) Small shriveled kernels (other kernels), which often had the only visible *A. flavus* in the sample and may have contained aflatoxin, were not included in the sample for aflatoxin analysis.

(iii) Not all isolates of *A. flavus* produce aflatoxin.

Failure to detect *A. flavus* in all of the samples containing aflatoxin may result from one or more of the following:

(i) Visible *A. flavus* growth often is found on less than 0.1 % of the kernels in farmers' stock peanuts later shown to contain aflatoxin. Therefore, due to sampling error, kernels with visible *A. flavus* may not always be included in 500-gram samples from contaminated peanuts.

(ii) *A. flavus* can grow and produce aflatoxin before it can be detected by visual inspection.

(iii) Fungi other than *A. flavus* might produce aflatoxin.

Use of larger samples and examination of loose-shelled kernels in the samples for visible *A. flavus* might increase the efficiency of detecting contaminated peanuts.

Although not 100 % accurate, examination for visible *A. flavus* on damaged kernels and other kernels in official grade samples from farmer's stock peanuts is a simple, effective method to detect peanuts which might contain large amounts of aflatoxin. The advantage of diverting peanuts that might be highly contaminated with aflatoxin to separate storage appears to far outweigh the disadvantage of diverting into separate storage some aflatoxin-free peanuts that have fungal growth resembling *A. flavus*.

References

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