Fluorescence in Pistachio Nuts Contaminated with Aflatoxin

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ABSTRACT

Bright greenish-yellow fluorescence under long wave ultraviolet light was observed on the shells of 7% of the nuts in samples from 46 aflatoxin contaminated commercial lots of Iranian pistachio nuts. Kernels from the fluorescent nuts contained 50% of the aflatoxin in the samples. No aflatoxin was found in any of the shells. When kernels and shells were cultured, toxicogenic fungi grew from only 4% of the shells and 21% of the kernels from fluorescent nuts and from 9% of the shells and 15% of the kernels from nonfluorescent nuts.

INTRODUCTION

Pistachio nuts imported into the US are tested for aflatoxin contamination (1). Although 24-kg samples are used for the tests, there is considerable variation among replicated aflatoxin test results from a contaminated lot. Apparently, a very small percentage of the nuts contain high concentrations of aflatoxin, and sampling errors in testing pistachio nuts for aflatoxin contamination are similar to those found for shelled peanuts (2). Grinding and testing large samples of pistachio nuts for aflatoxin is inconvenient and expensive. Improved procedures for detecting aflatoxin contamination and for removing aflatoxin contaminated nuts from commercial lots would benefit the entire pistachio industry and the consumer.

We observed that under long wave ultraviolet light (360 nm), shells of some pistachio nuts exhibit a bright greenish yellow (BGY) fluorescence similar to that reported for cottonseed and corn (3,4). Establishment of a correlation between aflatoxin contamination in pistachio nuts and BGY fluorescence of their shells would enable selective sampling of suspect nuts for chemical assay to insure detection of aflatoxin contamination in commercial lots and might justify removal of fluorescent nuts by electronic sorting or hand picking.

We have investigated the possible relationship between BGY fluorescence and aflatoxin contamination in pistachio nuts.

EXPERIMENTAL PROCEDURES

Samples were taken from 46 aflatoxin contaminated lots of Iranian pistachio nuts at the New York port of entry into the US. These samples were taken by the Agricultural Marketing Service, USDA, after previous tests had shown aflatoxin contamination in the lots. The samples weighed between 8-34 kg.

The samples were examined under long wave ultraviolet light (UVL-21 lamps, Ultra-Violet Products, Inc., San Gabriel, CA), and nuts with BGY fluorescing shells were removed by hand. Because fluorescence was not uniformly distributed, it was necessary to examine the entire surface of each nut. Nuts with very low levels or questionable BGY fluorescence were included with the fluorescent nuts.

All samples were shelled in a pistachio sheller designed by the Agricultural Research Service, USDA. (Shells constitute about 50% of pistachio nut weight). Each sample of shelled kernels then was mixed with an equal wt of oyster shells and ground for 3 min in a Hobart vertical cutter-mixer. A 200-g subsample of the ground mixture was analyzed for aflatoxin by the Waltking method for peanuts (5).

Both BGY fluorescent and nonfluorescent shells from samples of pistachio nuts with high levels of aflatoxin contamination were analyzed for aflatoxin. Shells from samples 39 through 46 (listed in Table I) were ground in a subsampling mill (6) with a 3/16-in. screen. A 300-g subsample of the shells from each sample was extracted and analyzed for aflatoxin by the Waltking method for peanuts (5).

To determine the kinds of fungi growing on the fluorescent and nonfluorescent nuts, 50-nut samples were taken from 7 commercial lots. The nuts were hand shelled and the 50 kernels and 100 half-shells from each sample were surface disinfected in 1% sodium hypochloride for 1 min, rinsed in sterile distilled water, and cultured on Czapek's agar plus 6% NaCl at 25 C. Beginning 4 days after isolation, the cultured parts were examined at 2 day intervals for 10 days. The fungi growing from these parts were either identified in the culture dishes or subcultured for later identification.

RESULTS

Sampling Error

Proper interpretation of the results from this study require an appreciation of the sampling errors related to aflatoxin tests. Whitaker, et al., (2) discussed errors in aflatoxin tests on shelled peanuts and gave the following equation for sampling variance σ_s^2:

$$\sigma_s^2 = (10{,}634/n)\,(9.0546\,\mu^{1.3955} - 0.3494\,\mu^{1.7867}), \qquad (I)$$

where n = number of kernels in the sample, and μ = parts per billion (ppb) aflatoxin concentration in the population sampled. The coefficient of variation (CV) due to sampling was computed by the following equation:

$$CV = 100\,\sigma_s/\mu. \qquad (II)$$

Our experience with aflatoxin tests on pistachio nuts indicated that sampling errors for pistachio nuts were similar to those for peanuts. Although subsampling errors and analytical errors also were involved, these errors were probably much smaller than sampling errors and for this study were ignored.

The above equations were used to calculate expected CVs for aflatoxin concentrations in 3 types of samples used in this study. These CVs are listed in Table II along with average values for n and μ used in the calculations. Expected CVs ranged from 82 to 164% for individual samples and from 12 to 24% for the averages of all 46 samples within each type. These values indicated extremely large sampling errors in aflatoxin determinations on individual samples, and that averages across all 46 samples contained considerable sampling error.

Aflatoxin Analyses

The weighted average aflatoxin concentration of kernels from fluorescent shells (FS kernels) and nonfluorescent shells (NFS kernels) within each sample ranged from 6 to 97 ppb and averaged 24 ppb (Table I). FS kernels constituted from 4 to 17% of the total kernels and averaged 7%. In all except 4 samples (samples 8, 12, 15, and 38), the aflatoxin concentration was higher in FS than in NFS kernels. The average aflatoxin concentration in the FS kernels was 150 ppb compared to only 14 ppb in the NFS kernels. An average 50% of the aflatoxin in the samples was

[1]Paper number 4670 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, NC 27607.

TABLE I

Aflatoxin and Wt Distribution Between Pistachio Kernels from BGY[a] Fluorescent and Nonfluorescent Shells

Sample Number[b]	Total sample of kernels		Kernels from fluorescent shells			Kernels from nonfluorescent shells		
	Wt (Kg)	Aflatoxin (ppb)	Total wt (%)	Aflatoxin (ppb)	Total aflatoxin (%)	Total wt (%)	Aflatoxin (ppb)	Total aflatoxin (%)
1	8	6	5	95	79	95	1	21
2	11	7	6	116	100	94	0	0
3	6	7	7	104	100	93	0	0
4	13	7	5	14	11	95	7	89
5	5	7	5	10	7	95	7	93
6	9	8	6	139	100	94	0	0
7	9	8	6	135	100	94	0	0
8	10	8	4	0	0	96	8	100
9	7	9	9	97	100	91	0	0
10	9	9	5	12	7	95	9	93
11	8	9	9	25	25	91	7	75
12	7	10	7	0	0	93	11	100
13	10	11	8	55	40	92	7	60
14	9	12	10	120	100	90	0	0
15	6	12	5	0	0	95	13	100
16	9	12	6	23	12	94	11	88
17	9	13	5	126	48	95	7	52
18	9	13	5	101	39	95	8	61
19	9	14	5	156	56	95	7	44
20	10	14	7	114	57	93	7	43
21	13	15	4	54	14	96	13	86
22	7	15	8	192	100	92	0	0
23	9	15	4	210	56	95	7	44
24	11	15	4	204	54	96	7	46
25	5	16	7	230	100	93	0	0
26	4	17	8	210	100	92	0	0
27	5	19	6	27	9	94	18	91
28	5	19	5	124	33	95	13	67
29	11	20	8	114	46	92	12	54
30	9	21	5	174	41	95	13	59
31	16	23	5	401	87	95	3	13
32	6	23	9	181	71	91	7	29
33	6	23	9	120	47	91	13	53
34	12	25	6	330	79	94	6	21
35	11	32	4	81	9	96	30	91
36	8	34	6	42	7	94	32	93
37	7	35	9	212	54	91	17	46
38	5	37	4	0	0	96	39	100
39	10	39	6	126	19	94	33	81
40	9	40	10	402	100	90	0	0
41	17	44	6	124	17	94	39	83
42	6	48	7	320	47	93	27	53
43	10	53	8	667	100	92	0	0
44	11	71	11	182	29	89	57	71
45	6	92	9	500	49	91	52	51
46	13	97	17	212	37	83	73	63
Average	9	24	7	150	50	93	14	50

[a]BGY = Bright greenish yellow.

[b]Samples ranked according to aflatoxin concentration.

TABLE II

Expected Coefficients of Variation (CV) Due to Sampling for Three Types of Samples

Type of sample	n[b]	μ	Individual test CV (%)	CV for average of 46 samples (%)
Kernels from BGY fluorescent shells	1,260	150	164	24
Kernels from nonfluorescent shells	16,740	14	102	15
All kernels	18,000	24	82	12

[a]Average number of kernels/sample (n) and average parts per billion (ppb) aflatoxin/sample (μ) were used to compute the expected CV for aflatoxin concentrations of individual samples within each type. This CV was divided by $\sqrt{46}$ to obtain the expected CV for the average of 46 samples.

[b]Average wt of pistachio kernels used in this study was 0.5 g.

in FS kernels, which constituted only 7% of the total kernels.

Because FS kernels from samples 8, 12, 15, and 38 tested negative for aflatoxin, not all fluorescent pistachio nuts contained aflatoxin. Studies on a large number of other samples in our laboratory have indicated that all lots of pistachio nuts contained fluorescent nuts, but these fluorescent nuts often tested negative for aflatoxin. Apparently, aflatoxin was not produced in the shells of pistachio nuts. Although aflatoxin in kernels from samples 39 through 46 averaged 60 ppb, none was detected in the shells from these samples.

Fungal Infection

The predominant fungi growing from the cultured ker-

TABLE III

Percentage of Surface Disinfected Pistachio Kernels and Their Shells which Yielded the Indicated Fungus when Cultured on Czapek's plus 6% NaCl Agar[a]

Sample Number	BGY Fluorescent Nuts								Nonfluorescent Nuts							
	Aspergillus niger (%)		*Aspergillus flavus* (%)		*Penicillium* sp. (%)		Other fungi[b] (%)		*Aspergillus niger* (%)		*Aspergillus flavus* (%)		*Penicillium* sp. (%)		Other fungi[b] (%)	
	K	S	K	S	K	S	K	S	K	S	K	S	K	S	K	S
47	100	79	13	1	13	0	3	17	52	18	33	9	2	0	2	0
48	44	27	56	0	5	2	5	0	16	10	3	0	9	2	9	11
49	100	44	4	2	27	5	2	5	33	25	8	3	0	4	4	6
50	74	36	16	2	0	8	4	0	54	16	14	4	6	2	14	0
51	98	50	26	20	28	8	2	0	84	44	20	10	26	6	4	0
52	76	44	8	0	42	10	0	0	60	22	14	16	16	10	0	0
53	90	40	26	6	2	8	4	0	28	40	14	18	6	8	6	4
Average	83	46	21	4	17	6	3	3	47	25	15	9	9	9	6	3

[a]50 kernels (K) and 100 half-shells (S) were cultured from each 50-nut sample.

[b]Other fungi included *Aspergillus repens*, other nontoxicogenic species of *Aspergillus*, and species of *Alternaria, Rhizopus,* and *Syncephalastrum.*

nels and shells were *Aspergillus niger, Aspergillus flavus,* and species of *Penicillium* (Table III). *Aspergillus repens*, other species of *Aspergillus* which do not produce aflatoxin, and species of *Alternaria, Rhizopus,* and *Syncephalastrum* also grew from some of the kernels and shells.

Other investigators (7) have reported that *A. flavus* isolated from BGY fluorescent cotton fibers produced kojic acid, which was converted to the BGY fluorescing substance by plant tissue peroxidase. Because *A. flavus* grew from only 21% of the FS kernels and 4% of the BGY fluorescent shells, it appeared that fungi other than *A. flavus* produced BGY fluorescence in pistachio shells. However, *A. flavus* could have grown in the kernels and shells, but have been nonviable at time of culture. The surface sterilization procedure may have killed the fungus, especially in the thin shells. The production of BGY fluorescent compounds probably was more closely related to the amount of fungal growth than to the presence of viable propagules; so growth of fungi from a large percentage of cultured kernels and shells from nonfluorescent nuts did not prove that growth of the fungi occurred without production of BGY fluorescent compounds, but only that viable propagules were present.

The fungal population found in this study agreed with previous observations made by one of us (J.W. Dickens, unpublished data). In 1972 pistachio nuts infected with *A. flavus* were found on the Amiri variety of pistachio trees in the experimental orchard of the Pest Control and Plant Diseases Research Institute at Rafsanjan, Iran. Tentative microscopic identification of *A. flavus*, by a procedure developed for peanuts (8), was supported by chemical assay of 2 badly molded nuts, which indicated an aflatoxin concentration > 21,000 ppb. The nuts apparently became infected after the husk and shell split during nut development. A greater number of other nuts with partially blackened, split husks and opened shells were infected with what appeared to be *A. niger* and species of *Penicillium*. Molding was most prevalent when the split in the husk and shell coincided. Examination under long wave ultraviolet light revealed BGY fluorescent shells on most of the molded nuts but not on the apparently sound nuts.

If nontoxicogenic fungi or other factors cause BGY fluorescence, this fluorescence is not a specific indication of the presence of toxicogenic molds. However, BGY fluorescence may indicate that conditions have been favorable for growth of toxicogenic molds on the pistachio nuts.

ACKNOWLEDGMENTS

Best Foods, Division of CPC International provided financial support. C.D. Latham and B.L. Joyner, Agricultural Marketing Service, USDA, assisted in processing samples and making aflatoxin analyses.

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[Received June 10, 1975]