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Aflatoxin Contamination in Dried Figs: Distribution and Association with Fluorescence

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The aflatoxin distribution was studied in a naturally contaminated batch of figs by analyzing more than 400 single figs and samples totalling over 90 kg. The situation closely resembles the pattern described for peanuts: only a very small number of figs were contaminated; the levels of contamination however were very high in individual fruits. The degree of contamination was estimated to be ca. 1 in 100. Evidence is presented in detail that bright greenish yellow (BGY) fluorescence under UV light (365 nm) is strongly correlated with the occurrence of aflatoxin contamination in a qualitative (but not in a quantitative) way. Removal of all BGY-fluorescent figs from a 56-kg sample effectively lowered the original contamination level from 22.6 to 0.3 ppb aflatoxin B_1. The sorting procedure is simple and fast and may easily be used to efficiently "clean" large batches of dried figs on an industrial scale before retail distribution. Microbiological investigations revealed the presence of aflatoxin-producing strains of *Aspergillus flavus* and *Aspergillus parasiticus*.

In 1985 the laboratory of a food retail chain reported that dried figs, imported from Turkey, were partly contaminated with aflatoxins. Various food-control laboratories in Switzerland subsequently increased their activities in analyzing figs for aflatoxin and reported the detection of tens of ppb's in samples (homogenates) of 2-5 kg. Single figs were found to contain up to 5 mg of aflatoxin B_1/kg (unpublished reports, Kantonales Laboratorium of Basle). On the basis of these findings, the Federal Office of Public Health set the legal limit for aflatoxins on figs to the same levels as it is for nuts; i.e. 1 ng/g of AFB_1 and 5 ng/g for the sum of toxins B_1, B_2, G_1, and G_2.

The occurrence of aflatoxin in figs was observed several years ago (*Food Chem. News*, 1974), and aflatoxin production during ripening was studied by Buchanan et al. (1975). Other carbohydrate-rich fruit, such as pineapple and cooked apricot, were recognized as good substrates for aflatoxin production by Morton et al. (1979). Figs had not been recognized as a high-risk commodity in Switzerland, and the distribution of aflatoxin contamination in figs does not seem to be known. The existing sampling plans (Campbell et al., 1986) apparently have been derived by extrapolating from the situation found in peanuts.

With contaminated figs of uniform origin, we studied the distribution of aflatoxin within the batch, the association of aflatoxin with toxin-producing *Aspergillus* species, and the association of bright greenish yellow (BGY) fluorescence with aflatoxin occurrence on individual figs.

MATERIALS AND METHODS

Figs. The average weight of dried Turkish figs (1985 crop) used for this work was 22.3 g (CV = 17.2%; n = 189). This weight was used to calculate the number of figs in the analyzed samples.

Aflatoxin Standard Solution. Aflatoxins B_1, G_1, B_2, and G_2 were obtained from Sigma Chemical Co. A solution in toluene/acetonitrile (98:2), containing 0.5 mg/L of each toxin, was prepared and assayed according to AOAC Method 26.009 (Horwitz, 1980).

Detection of BGY Fluorescence. A Blak Ray longwave ultraviolet lamp (Model B-100 A; 360 nm) was used. During the sorting and inspecting of figs, UV-protective spectacles, polyethylene gloves, and dust-protective facial masks were worn.

Color Slides. These were taken on Fujichrome 400 ASA with a Minolta XE-1 camera (lens 1:3.5, f = 100 mm).

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A Cokin-A 173 filter was used in series with a polarization filter. This combination was adjusted to the position transparent for red light. The figs to be photographed were illuminated with a Blak Ray lamp (see above; aperture 11, exposure time 3 min).

Extraction of Figs. Samples up to 5 kg were cut and homogenized in a 25-L meat cutter; 80 g of the homogenate was placed in a Waring Blendor. Single figs were also homogenized and extracted (see below) in a Waring Blendor. In its essence, the analytical procedure corresponded to the Official Swiss Method, published by the Working Group on Toxins (*Mitt. Geb. Lebensmittelunters. Hyg.*, 1982).

Methanol (200 mL; 100 mL for single figs) and water (20 mL; 10 mL for single figs) were added to the Blendor, and the mixture was homogenized for 3 min. After addition of 60 mL of water (30 mL in the case of single figs) the slurry was again homogenized for 3 min. The resulting mixture was filtered through fluted filter paper, and 70 mL of filtrate was collected and transferred into a separatory funnel. Water (20 mL) and dichloromethane (90 mL) were added, the mixture was shaken, the organic phase was drained off and dried with anhydrous sodium sulfate, and the solvent was evaporated on a rotary evaporator (ca. 20 Torr, 40 °C). The residue was redissolved in 1 mL of toluene/acetonitrile (98:2); this solution was used for thin-layer chromatographic analysis.

Isolation and Cultivation of Molds. From a homogenized batch of figs, 10 g was suspended in 90 mL of peptone water containing 8.5 g of NaCl and 1 g of Trypticase Peptone (BBL 11921) in 1 L of water. An aliquot (0.1 mL) of this suspension was incubated as a surface culture on malt extract agar (Merck No. 5397) for 7 days at 25 °C.

Single colonies were isolated and subcolonies obtained again on malt extract agar. Uniform colonies were characterized microscopically. Single-fig homogenates were surface-cultured directly on malt extract agar.

Colonies of Aspergilli, isolated as described above, were suspended in 200 mL of a broth, containing 3% yeast extract (BBL 11456) and 10% glucose. After incubation for 2 weeks at 25 °C the culture filtrates were analyzed for aflatoxin.

Extraction of Cultivated Molds. To culture broth (10 mL, filtered) were added 1 g of NaCl and 10 mL of water. This mixture was extracted once with 50 mL and once with 40 mL of dichloromethane in succession. The combined organic phases were treated further as above.

Thin-Layer Chromatography and Quantitation. The extracts to be assayed and the respective standard solutions were spotted on a line ca. 12 cm from the bottom of a commercial silica TLC plate (aluminum sheets, Merck No. 5553) activated immediately before use for 30 min at 100 °C, and the first development was carried out in anhydrous diethyl ether over the whole distance. The portion of the plate from ca. 13.5 cm upward, containing impurities, was cut off, and the plate was redeveloped in the opposite direction with use of a mixture of chloroform/acetone/water (88:12:0.2). The chromatograms were quantitated with a fluorodensitometer (CAMAG-TLC-scanner Mk. II) and an excitation wavelength of 365 nm and a 400-nm cutt-off filter on the emission side. The results are not corrected for recoveries, which were, at a spiking level of 1.25 ppb, regularly between 70 and 90%. The limit of detection was 0.2 ppb for each toxin.

RESULTS AND DISCUSSION

Distribution of Aflatoxins. Several samples were taken from a batch of 160 kg of dried figs and analyzed for aflatoxins. The results shown in Table I indicate a heterogeneous but rather high contamination.

Table I. Aflatoxin in Dried Figs (Crop 1985, Turkey)

sample wt, g	no. of figs (approx)	aflatoxin, ng/g B_1	aflatoxin, ng/g G_1
4800	215	0.4	0.4
3600	161	0.9	0.9
5200	233	0.7	0.3
6400	287	13.4	15.0
5200	233	22.8	47.7
4800	215	37.0	22.0
30000[b]	1345[c]	13.0[a]	15.2

[a] Mean value. [b] Total weight. [c] Total number of figs.

Table II. Aflatoxin in 386 Randomly Selected Figs[a]

aflatoxin concn, ng/g	no. of figs with aflatoxin B_1	no. of figs with aflatoxin G_1
<0.2	292	337
0.2–1	48	28
1–5	37	17
5–10	7	2
10–20	1	0
20–30	1	2
av	0.5	0.3

[a] Total weight ca. 8600 g.

Table III. Aflatoxin in 16 Dark (Discolored) Figs

aflatoxin concn, ng/g	no. of figs with aflatoxin B_1	no. of figs with aflatoxin G_1
<0.2	8	13
0.2–1	3	1
1–5	3	2
5–10	1	0
10–20	1	0

Obviously, two conclusions may be drawn from these analyses, concerning the specific lot in question: (a) The degree of contamination in single samples with a weight of ca. 5 kg does not give representative results. (b) The lot probably contains a few very highly contaminated figs.

(a) Recognition of Contaminated Figs: Random Selection. In order to obtain some information on the distribution of the contamination and the level of aflatoxin concentration in contaminated fruits, 386 randomly selected figs (equivalent to 8.6 kg) were analyzed individually. The results in Table II show that a large majority of figs are virtually free of contamination. The few aflatoxin-containing fruits, however, did not account fully for the levels found in some of the samples (Table I), and the suspicion remained that there were figs in the batch with a high level of contamination. Therefore, subsequent efforts were directed toward finding a macroscopically observable parameter that would identify contaminated figs.

(b) Recognition of Contaminated Figs: Dark Discoloration. A number of dark, discolored figs were observed in the lot. These figs were sorted out and analyzed individually to determine whether this discoloration was associated with aflatoxin contamination. The results (Table III) show that this was not the case to a high degree.

(c) Recognition of Contaminated Figs: BGY Fluorescence. BGY fluorescence associated with aflatoxin concentration in the milligram/kilogram range was observed in cotton (Ashworth and McMeans, 1966; Marsh et al., 1969) and in corn (Shotwell et al., 1972, 1974; Fennell et al., 1973). To determine whether a similar correlation existed in figs, ca. 2500 individual fruits were inspected

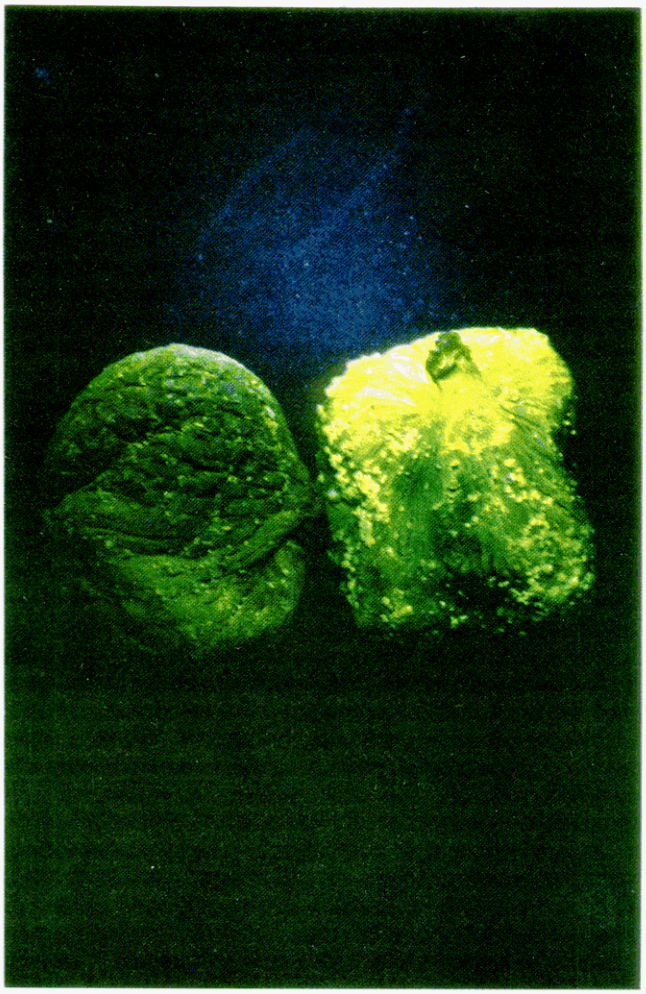

Figure 1. Figs under UV light (360 nm), one of them showing typical BGY fluorescence. Only the fluorescent fruit was aflatoxin contaminated.

Table IV. Aflatoxin in 62 Figs Showing BGY Fluorescence

aflatoxin concn, ng/g	no. of figs with aflatoxin	
	B_1	G_1
<0.2	10	41
0.2–1	4	2
1–10	14	5
10–100	8	4
100–1000	14	4
1000–10000	11	4
>10000	1	2

for BGY fluorscence and 62 figs showing fluorescence of varying area size and intensity were found and analyzed for their aflatoxin content. Figure 1 shows an example of a strongly fluorescent fig. The results in Table IV show clearly that there is indeed a strong association of BGY fluorescence with aflatoxin contamination. However, the intensity of the fluorescence was not indicative for the concentration of aflatoxin.

Having successfully isolated a number of highly contaminated figs by BGY fluorescence, the degree of the contamination in the remaining "purified" figs was determined. The results are listed in Table V and give convincing evidence that most of the highly contaminated fruits had been eliminated, i.e. were among the 62 fluorescent figs.

Fluorescence was not restricted to the surface: most BGYF-positive fruits showed fluorescence throughout the fig. To determine whether not only fluorescence but also

Table V. Analysis of "Sorted" Figs after Elimination of 62 BGY-Fluorescent Fruits

sample wt, g	no. of figs (approx)	aflatoxin, ng/g	
		B_1	G_1
5000	224	0.4	0.4
5000	224	0.3	0.3
5000	224	0.4	0.3
5000	224	0.7	0.4
5000	224	0.3	0.3
5000	224	0.5	<0.2
5000	224	<0.2	<0.2
5000	224	0.4	0.8
5000	224	0.2	<0.2
5000	224	0.3	<0.2
5000	224	0.3	0.6
55000[b]	2464[c]	0.3[a]	0.3

[a] Mean value. [b] Total weight. [c] Total number of figs.

Table VI. Aflatoxin in Five Individual Fluorescent Figs

fig no.	aflatoxin, ng/g			
	fluorescent		nonfluorescent	
	B_1	G_1	B_1	G_1
1	170	430	9	29
2	290	20	3	0.3
3	1400	<0.2	8	<0.2
4	540	1900	3	24
5	100	480	33	77

Table VII. Fungi Isolated from Figs (%)

	selection criteria		
	randomly selected	dark	BGY fluorescent
total no. of figs investigated	173 (=100%)	16 (=100%)	43 (=100%)
fungal growth observed (%)	22	62	63
Aspergillus species	10	38	28
A. flavus or *A. parasiticus*	3	6	19
aflatoxin; no *A. flavus*/para.	26	44	63
A. flavus/para.; no aflatoxin	1	6	2
aflatoxin; *A. flavus*/para.	2	0	16

aflatoxin was distributed evenly in contaminated figs, five fluorescent fruits were cut in two: one piece containing the entire fluorescent part of the surface and the other exhibiting no fluorescence. The results in Table VI show that, unlike fluorescence as such, aflatoxin was localized in defined, apparently rather small compartments within a fruit and associated with *surface* fluorescence in particular.

On the basis of these findings and on the observation that figs packed in small boxes for retail sale are usually in very close contact with each other, it was of interest to study the question of cross-contamination among figs. Fruits packed in direct contact with the fluorescent parts of two highly contaminated figs (1400 and 7100 ng of aflatoxin B_1/g) showed only low contamination (31 and 25 ng/g, respectively); figs in contact with the nonfluorescent parts of one of the contaminated fruit (7100 ng/g) showed virtually no contamination (2 and 3 ng/g). This indicates that cross-contamination in packed figs plays a minor role.

Fungal Growth and Aflatoxin Contamination. Dark figs (Table III), some of the fluorescent figs (Table IV), and some of the randomely selected figs (Table II) were examined for the presence of fungal spores by the cultivation methods explained in Materials and Methods. *Aspergillus flavus* and *Aspergillus parasiticus* could be

identified in several instances, and in very rare cases *Aspergillus fumigatus* and *Aspergillus niger* were isolated; the results are summarized in Table VII. Fluorescent figs showed the highest percentage of *Aspergillus* growth, although the absolute amount of fruits on which intact spores of the specific aflatoxin-producing fungi could be found was rather low.

Two strains of *A. parasiticus* were isolated from a homogenized sample of figs and examined for aflatoxin production. The two strains exhibited markedly different behavior: whereas one strain produced comparable amounts of aflatoxins B_1 and G_1 (3.8 and 5.7 μg/mL culture broth, respectively), the other strain produced almost exclusively aflatoxin B_1 (5.6 μg/mL culture broth; aflatoxin G_1 <0.2 μg/mL). The toxin-producing properties of the isolated fungi always matched the toxin pattern found on the respective fruits.

Aflatoxin Contamination and Fluorescence. BGY fluorescence in cotton and other plants was shown to result from the transformation of kojic acid by a peroxidase-type reaction (Marsh et al., 1969). Kojic acid however is not a unique metabolite of *A. flavus* and *A. parasiticus*; several other *Aspergillus* and *Penicillium* species are known to produce this acid. Moreover, not all *A. flavus* strains that show kojic acid production also produce aflatoxin; but, on the other hand, all aflatoxin-producing strains investigated by Parrish et al. produced kojic acid. The association of BGY fluorescence with aflatoxin occurrence is therefore dependent on the fungal population. This situation is at present not known for figs. On cotton fiber at harvest, Marsh et al. rarely observed (1969) any kojic acid producing fungi other than *A. flavus* and *A. parasiticus*.

Although high concentrations of aflatoxins were found in fluorescent cotton and corn, not all aflatoxin-positive seeds or maize kernels exhibited fluorescence. The failure of cotton fiber to produce BGY fluorescence when infected with *A. flavus* was described by Ashworth and McMeans (1966) as well as by Marsh et al. (1969). The lack of BGY fluorescence in corn that contained 3 mg of aflatoxin/kg was reported by Fennell et al. (1973), while Shotwell et al. (1972, 1974) found high aflatoxin concentrations in fluorescent kernels.

As can be deduced from Table V, the procedure of eliminating all fluorescent figs did not at the same time remove all aflatoxin-containing fruits. Obviously, there remained still a number of contaminated figs in the sorted lot. If the contamination of the 11 individual subsamples would have been localized on 11 figs, those particular fruits would then have contained between 1000 and 3500 ng of aflatoxin B_1 or for a fig weighing 22.3 g, between 45 and 160 ng/g. This corresponds reasonably well with the contamination pattern found in the randomly selected figs discussed in Table II.

An explanation for the presence of contaminated figs without fluorescence might be found in the following arguments:

1. BGY fluorescence on locks of cotton showed a marked decrease of intensity after direct exposure to sunlight (Marsh et al., 1969). A similar experiment with a fluorescent fig exposed to intensive sunlight for 4 h resulted in a marked loss of fluorescence.

2. In order to test whether rain might wash off fluorescent material, a fluorescent fig was held under a stream of tap water for 6 min. This treatment also resulted in a loss of fluorescence, albeit not as strongly as was observed with light.

3. A few figs showed only very weak fluorescence on the outside with intensively fluorescent inner parts.

4. The occurrence of *Aspergillus* strains producing aflatoxin but no kojic acid cannot be ruled out.

Despite the fact that BGY fluorescence sorting seems to miss some contaminated figs, the probability to efficiently "clean" a batch of figs is very high, as is obvious from Table V.

Aflatoxin Contamination and Infection. Buchanan et al. (1975) studied the growth of *A. flavus* on figs and the aflatoxin production in various stages. When green fruits were inoculated (10^5 conidia *A. flavus* in 0.1 mL of water), those fruits taking longest to ripen had the highest aflatoxin content (72 mg/kg). Fruits inoculated in the firm-ripe state showed very little aflatoxin if dried immediately; the authors observed an increased aflatoxin content with increasing drying time.

In Turkey, the figs are left on the trees until they are shrivelled ripe. They then fall to the ground, which occasionally is covered with cloths. The fruits are dried further in the sunlight before being collected. Obviously, these conditions seem to be favorable for aflatoxin production in infected fruits.

The mechanisms of natural infection of figs by *A. flavus* and *A. parasiticus* remain unclear. A few figs among the many fruits inspected showed internal fluorescence only. This fact points, in our opinion, to infection of these fruits at an early stage in their developement. The vast majority of the aflatoxin-containing figs showed external fluorescence as well as internal. The aflatoxin contamination was restricted to a great extent to that part of the fig showing surface fluorescence. It is concluded that infection and/or development of the fungus must have taken place in a rather late stage of the ripening process, perhaps only after the fruits had fallen from the trees and had been lying on the ground to dry.

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Registry No. Aflatoxin B_1, 1162-65-8; aflatoxin G_1, 1165-39-5.

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