Short Communication

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Bright Greenish Yellow Fluorescence and Aflatoxins

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Contamination of oil seeds with aflatoxins may cause hazards to animals and human beings through contamination of poultry products and the milk of milch cattle. This study was undertaken to design a method to detect aflatoxin contamination by a simple and rapid test i.e. BGYF test or black light technique.

Aspergillus flavus, A. parasiticus and the recently described species *A. nomius* are recognized as the sole producers of aflatoxins i.e. a group of carcinogenic metabolites [1,2]. Presence of a bright greenish yellow fluorescence (BGYF) on cotton lint viewed under long wave ultraviolet light has been associated with invasion of the bolls by *A. flavus* [3,4].

Aflatoxins and BGYF material were extracted by using prepared phenyl columns [5]. The aqueous phase containing the BGYF material was dried on a rotary evaporator and the residue was redissolved in methanol. The chloroform phase was dried under a nitrogen stream.

Aflatoxins were determined and quantified by simple TLC and 2-dimensional TLC and confirmed by spraying with 50% H_2SO_4. They were quantified by comparison with known standards [6]. Standard BGYF material and extracted BGYF material were dissolved in butanol and added to the prepared columns. BGYF material was eluted by adding butanol : acetic acid : water in a ratio of 3:1:1 under N_2 pressure (15 PSI) and 10 fractions of 5 ml each were collected.

10 µl from each fraction of eluted standard BGYF and sample extracts were spotted on the cellulose plates and developed with butanol : acetic acid : water (3:1:1). Numbers of BGY fluorescent kernels and their respective weights are shown in Tables 1 - 2. The maximum concentration of aflatoxins B_1 and B_2 was in sample 5A, being 24096 and 2560 µg kg^{-1} respectively, which corresponded to the highest number of BGYF kernels (60) with maximum effective weight (0.83 g). There was a correlation between the aflatoxin contents and the weight of kernels with BGYF in most of the cases. However, in some cases this relationship did not exist, for example, sample 6A having 0.60g affective weight of BGYF kernels had an aflatoxin content of 12500 µg kg^{-1} of B_1 while samples 3A and 1a both with effective weight of 0.62 g were found to contain 1209 µg kg^{-1} and 1129 µg kg^{-1} of B_1 respectively (Table 1). This difference was due to the fact that fluorescent kernels regardless of their degree of fluorescence were picked up, so BGYF count is not quantitative measure of aflatoxin in cotton seeds.

Cotton seed samples in batch B were found to have aflatoxin B_1 contents from 275 to 598 µg kg^{-1} and aflatoxin B_2 contents from 50 to 166 µg kg^{-1} (Table 2) indicating a positive, but not quantitative, relationship between BGYF kernels and aflatoxin level. In batch B aflatoxin concentration

TABLE 1. RELATIONSHIP BETWEEN BGY FLUORESCENT KERNELS* AND AFLATOXIN PRODUCTION IN NATURALLY CONTAMINATED COTTON-SEED SAMPLES.

Sample	No. of BGYF kernels (kg^{-1})	Weight of BGYF kernels (g kg^{-1})	Effective weight** (g)	Aflatoxins produced (µg kg^{-1}) B_1	B_2
1A	54	4.43	0.62	1129	137
2A	52	4.23	0.56	933	156
3A	42	3.43	0.62	1209	37
4A	57	4.02	0.59	1031	250
5A	60	4.65	0.83	24096	2560
6A	41	3.38	0.60	12500	1180
7A	53	3.99	0.70	2394	516
8A	51	3.98	0.71	2976	1012

*BGY Fluorescent kernels only were analysed for aflatoxins.
**Weight in grams of original sample contained in final extract.

TABLE 2. RELATIONSHIP BETWEEN BGY FLUORESCENT KERNELS* AND AFLATOXIN PRODUCTION IN NATURALLY CONTAMINATED COTTON SEED SAMPLES.

Sample	No. of BGYF kernels (kg^{-1})	Effective weight** (g)	Aflatoxins produced (µg kg^{-1}) B_1	B_2
1B	70	1	598	100
2B	55	"	411	75
3B	65	"	316	166
4B	64	"	467	137
5B	58	"	426	137
6B	40	"	275	50
7B	52	"	316	125
8B	56	"	366	100

*BGY Fluorescent kernels counted in one kilogram sample then mixed back.
**Weight in grams of original sample contained in final extract.

was lower than in the sample where BGYF kernels were extracted.

Spotting of purified BGYF material from cotton seed samples and BGYF reaction product produced *in vitro* (Standard) showed the BGY fluorescent spots at the same Rf of 0.40 [7]. Results of spotting of standard BGYF material from flash chromatography showed the highest fluorescence intensity in fraction 5 (Fig. 1). Yellow spots at the comparable Rf. of kojic acid were visible in long wave (366 nm) UV light. This yellow spot was present in fractions 1,2,3,4, but was not visible in the 5th fraction where BGY Fluorescence was visible.

Spotting of BGYF material from cotton-seed purified by flash column chromatography gave similar results to the standard but in fractions 2-5, the kojic acid spot was not visible. Thus it can be concluded that all the kojic acid was utilized to produce BGYF. The BGY Fluorescent spot was present between a blue spot at the base and a black spot at the top (Fig. 2). Black and blue spots were more prominent in fraction 4, where BGYF spot was not intense. These two additional spots were not present in the standard BGYF product. The black spot may be due to a derivative of kojic acid which has lower Rf and blue spot may be a precursor of BGYF material because it is just below the BGYF spot. Further studies are needed to elaborate the identity of these two spots.

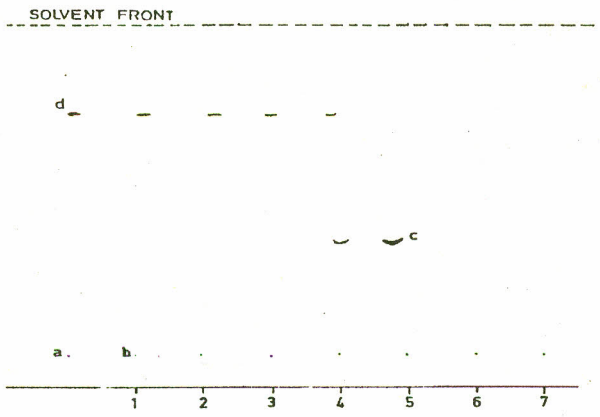

Fig. 1. Development of BGYF material (Standard) on TLC plate (cellulose coated) in butanol:acetic acid:water (3:1:1). a) Kojic acid (10µ lit spot), b) (1-7 fractions) BGYF extract (10µ lit spot), c) BGYF spot (developed) Rf. 40, d) Kojic acid spot (developed) Rf. 75.

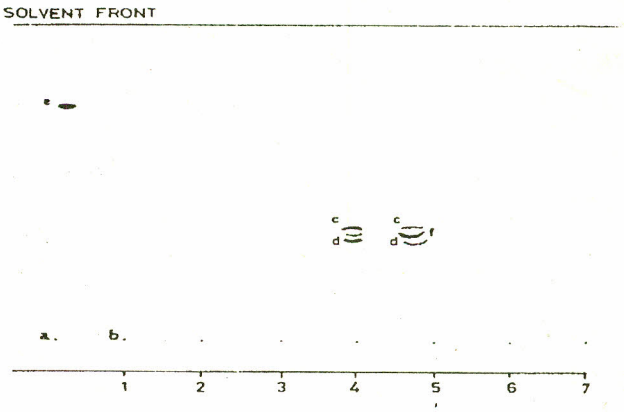

Fig. 2. Development of BGYF material (extracted from cotton seed) on TLC plate (cellulose coated) in butanol:acetic acid:water (3:1:1). a) Kojic acid (10µ lit spot), b) (1-7 fractions) BGYF extract (10µ lit spot), c) Black spot developed, d) Blue spot developed, e) Kojic acid (developed) Rf. 75, f) BGYF spot (developed) Rf. 40.

Key words: Aflatoxin detection, Bright greenish yellow fluorescence.

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