Treatment of Peanuts with Dimethyl Sulfoxide and its Effect on Aflatoxin Production by Aspergillus flavus

G. W. Rambo and G. A. Bean

Former graduate student and Associate Professor, respectively, Botany Department, University of Maryland, College Park 20742.

Present address of senior author: Department of Life Sciences, Purdue University, Lafayette, Indiana 47907.

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ABSTRACT

Peanut seeds (cultivar 'Early Runner') were treated with different concentrations of dimethyl sulfoxide (DMSO) to study its effect on the production of aflatoxins by *Aspergillus flavus*. Treatment of peanut seeds with 2.5% DMSO or higher concentration prior to inoculation with *A. flavus*, caused an inhibition of normal conidial pigmentation and approximately 62%-64% inhibition in aflatoxin production.

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Studies on aflatoxin control have concentrated on either the detoxification of aflatoxin or control of the organisms producing aflatoxins (4,5). The treatment of plant tissue to prevent formation of aflatoxins by *Aspergillus* spp. has received little attention. In 1967, Davis & Diener (3) found that p-aminobenzoic acid (PABA) inhibited both production of aflatoxins and growth of *Aspergillus parasiticus* var. *globosum* Speare in liquid culture. They also reported that aflatoxin production was reduced 50% in peanuts soaked in PABA and reduced 30% in peanuts sprayed with PABA.

In 1969, Bean et al. (2) reported that aflatoxin-producing strains of *A. flavus* Link grown on medium containing dimethyl sulfoxide (DMSO), produced white instead of normal green conidia. They also reported that white conidia were killed more rapidly by exposure to ultraviolet irradiation than were green conidia. In 1971,

TABLE 1. Aflatoxin production by *Aspergillus flavus* after 7 days' growth on shelled or unshelled peanut seeds (cultivar 'Early Runner') soaked 30 min with dimethyl sulfoxide (DMSO) before inoculation

Treatment % DMSO	Aflatoxin produced (μg per culture)	
	Shelled peanut seeds	Unshelled peanut seeds
0	104[x] ab	342 ab
0.625	235 a	452 a
1.25	87 b	282 bc
2.5	40 b	124 cd
5.0	24 b	48 d
10.0	12 b	26 d
20.0	10 b	28 d

[x] Values represent avg μg aflatoxin in three cultures. Values followed by the same letters are not significantly different at the 1% levels for Duncan's multiple range test.

the same authors reported that increasing levels of DMSO in liquid medium reduced aflatoxin levels whereas the growth rate of the fungus was decreased only slightly (1). We have investigated the possibility of treating plant tissue (i.e., peanuts) with DMSO to determine whether this could be an effective, alternative method of controlling aflatoxin production.

MATERIALS AND METHODS.—Peanuts (*Arachis hypogaea* L., cultivars 'Early Runner' and 'Florigiant') were obtained from the United States Department of Agriculture Research Station, Tifton, Georgia. To investigate the influence of DMSO on the level of aflatoxins produced in peanuts inoculated with *A. flavus*, 50-g lots of shelled or unshelled Early Runner peanuts were placed in a 500-ml Erlenmeyer flask and soaked 30 min in 100 ml of 0, 0.625, 1.25, 2.5, 5.0, 10.0, or 20.0% DMSO in distilled water with 0.25% Triton X-100 surfactant. The DMSO solution was poured off, and the flasks were autoclaved for 3 min at 120 C and 1.27 kg/cm^2 (18 psi) to prevent germination of the peanuts and then cooled. A spore suspension (0.2 ml) of an aflatoxin-producing isolate of *A. flavus* (obtained from R. Welty, North Carolina State University, Raleigh) was added to each flask. The flasks were incubated for 7 days at 24 C.

The peanut kernels and shells were extracted for aflatoxins using methods outlined by Pons et al. (7). The peanut extracts and aflatoxin standards (obtained from Dr. Leo Goldblatt, Southern Utilization Research Laboratory, New Orleans, Louisiana) were spotted on a 0.25-mm Absorbosil thin-layer chromatographic plate and developed in a chloroform-methanol solvent system (97:3, v/v). The plates were dried and examined for fluorescence under short wave ultraviolet light in a Chromato-Vue chamber. Extracts also were analyzed on a Bausch & Lomb model 600 spectrophotometer at 362 nm to determine aflatoxin quantities.

The presence of aflatoxins was also verified by chick embryo bioassay, according to the method of McLaughlin et al. (6). The extracts were dried, redissolved in 70% ethanol, and 0.2 ml each of the peanut extracts, aflatoxin standard, or solutions of 0, 0.625, 5.0, 10.0 and 20.0% DMSO were injected into the air sac. Two-tenths ml of 70% ethanol was injected as the control. The eggs were incubated for 21 days and periodically candled to determine the percentage mortality. The bioassay was replicated three times and 20 eggs were inoculated for each treatment.

RESULTS.—*Growth and aflatoxin production by A. flavus in DMSO-treated peanut seeds.*—Mycelium of *A. flavus* was first observed 3 days after inoculation of shelled or unshelled peanuts. After 7 days, mycelial growth had completely covered the peanut kernels, except those treated with solutions of 5.0, 10.0, and 20.0% DMSO. Pigmentation of the conidia changed from a normal green color to a pale green, then white, with increased dosage of DMSO. This observation was reported previously (2).

When chloroform extracts from inoculated peanuts were examined by thin layer chromatography, aflatoxins B_1 and G_1 were detected in extracts from peanuts treated with 10.0 or 20.0% DMSO. Aflatoxins B_2 and G_2 were not detected in extracts from peanuts treated with 5.0, 10.0 or 20.0% DMSO. Aflatoxins were not detected in the peanut shells at any level of DMSO.

When the same chloroform extracts were examined spectrophotometrically, aflatoxins were recorded at all levels of DMSO treatment (Table 1). A concentration of 0.625% DMSO caused a slight increase in total aflatoxin level; treatment with higher DMSO concentrations decreased the level of aflatoxin. Extracts from unshelled and shelled peanuts treated with 2.5% solution of DMSO contained approximately 62-64% less aflatoxins than did extracts from peanuts treated with water.

Chick embryo bioassay.—The mortality rates of chick embryos injected with 0.2 ml of either the standard (.026 μg) or extracts from peanuts and *A. flavus* cultures treated with 0, 1.25, or 5.0% DMSO were greater than 90%. However, eggs injected with extracts from peanuts treated with 10% DMSO before inoculation had a mortality rate equal to that of the controls; i.e., 25%. Some of the chicks that hatched from the treatments of 10% DMSO or less had malformations that would probably have led to mortality.

DISCUSSION.—Prevention of formation of aflatoxins in agricultural crops is difficult because of residue problems with antifungal agents, incomplete control of the organism, or the expense involved (4,5). The most effective approach in aflatoxin control has been to prevent aflatoxin production by reducing the moisture content to a level that prevents growth of the organism (4,5). Since this measure is not always possible for peanuts, alternative methods, such as treatment with DMSO or PABA to prevent aflatoxin production should be considered.

This is the first report of inhibition of aflatoxin production by DMSO in non-living plant tissue. Possibly DMSO could also be used to inhibit aflatoxin production by treating peanuts immediately after harvest or prior to processing them provided that extensive testing indicates mammalian safety and market quality of the treated peanuts are not impaired.

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