Dimethyl Sulfoxide Inhibition of Aflatoxin Synthesis by Aspergillus flavus

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ABSTRACT

Aspergillus flavus was grown at 22, 28, and 35 C in a medium containing 0 to 50,000 ppm dimethyl sulfoxide (DMSO). Aflatoxin concentration and mycelial production were measured periodically for 6 weeks. Without DMSO in the medium, the optimum temperature for aflatoxin production was 35 C after 1 week, 28 C after 2 weeks, and 22 C after 4 and 6 weeks. The addition of 5,000 ppm DMSO caused a slight increase in aflatoxin concentration at 22 and 28 C after 1 week, at 35 C after 2 weeks, and at 28 C after 4 weeks. At all other temperatures and time intervals, aflatoxin concentration decreased with increasing levels of DMSO. The dry weight of mycelium decreased slightly at all temperatures with increasing DMSO concentrations. Phytopathology 61:380-382.

Aflatoxins are toxic, carcinogenic metabolites produced by members of the *Aspergillus flavus* group of fungi (15). The chemistry of aflatoxin and their effects on biological systems has been reviewed by Goldblatt (6).

When moisture and temperature are favorable, many agricultural products can be utilized as substrates by aflatoxin-producing fungi (11). Aflatoxin production can be prevented by partially drying the grain or oilseeds before storage or by storage under atmospheric conditions unfavorable for fungal growth (7). With some commodities, such as peanuts and cotton seeds, mold growth is stimulated by the subtropical conditions at time of harvest. Results from the use of chemicals to control storage fungi have been conflicting (7).

Production of aflatoxins in vitro can be prevented by including barium in the growth medium (8). Davis & Diener (4) reported that *p*-amino benzoic acid, potassium sulfite, and potassium fluoride prevented aflatoxin synthesis; however, only potassium sulfite inhibited aflatoxin production without reducing fungal growth.

Bean et al. (2) recently reported that aflatoxin-producing strains of *A. flavus* grown on medium containing dimethyl sulfoxide (DMSO), or certain other sulfur-containing compounds, produced white instead of the normal green conidia. When exposed to ultraviolet irradiation, the white conidia were killed more rapidly than green conidia. This paper reports the influence of DMSO on growth and aflatoxin production by *A. flavus*.

MATERIALS AND METHODS.—*Aspergillus flavus* Link ex Fries (ATCC 15517) used in these studies was cultured in 250-ml Erlenmeyer flasks each containing 50 ml of a medium made of 20 g sucrose, 0.5 g $MgSO_4 \cdot 7H_2O$, 3 g KNO_3, and 7 g yeast extract in 1 liter of water at pH 5.5 (5). The DMSO was added aseptically after the medium had been autoclaved. A spore suspension of the fungus was added to each flask, and the cultures were incubated at 22, 28, and 35 C without shaking. Each treatment was replicated at least 3 times.

The influence of DMSO on mycelium production was determined by collecting mycelium on preweighed filter papers which were then dried at 100 C for 24 hr and reweighed. For aflatoxin determination, the culture filtrate was extracted with chloroform, and the extract reduced to low volume and separated by thin-layer chromatography (12). Aflatoxin zones were eluted from the chromatograms, and concentrations were determined by calculation using the molar extinction coefficient (MEQ) of 21,800 at 362 nm, as reported for aflatoxin B (1, 3).

RESULTS.—Table 1 indicates the effect of DMSO on mycelial growth and aflatoxin production by *A. flavus* after 7 days. Most mycelium was produced at 35 C. The addition of 5,000 ppm DMSO caused a slight increase in aflatoxin production at 22 and 28 C. Cultures incubated at 35 C had less aflatoxin than the control at all concentrations of DMSO. Higher concn of DMSO, however, caused corresponding decreases in aflatoxin levels. The dry wt of mycelium at all temperatures decreased slightly with increasing DMSO levels.

Previous studies indicate that temperature influence varies with time as well as with isolate of *A. flavus* (13). The reduction in aflatoxin levels with increasing DMSO concentrations, therefore, could result from delayed production rather than from inhibition. The experiment was repeated and aflatoxin level and mycelial production were determined after 2, 4, and 6 weeks.

Maximum growth of *A. flavus* occurred at 22 C at all three time intervals. The presence of 5,000 ppm DMSO increased mycelium production at all three temperatures after 4 weeks, and at 22 and 28 C after 6 weeks. At all other temperatures or time intervals, DMSO concentrations of 5.000 ppm or more caused a decrease in mycelium production.

Aflatoxin production after 2, 4, and 6 weeks in the presence of DMSO is summarized in Fig. 1. Without DMSO in the medium, the optimum temperature for aflatoxin production was 28 C after 2 weeks, but 22 C after 4 and 6 weeks. Previous workers report that the optimum temperatures for growth do not coincide with those for aflatoxin production (13). Of greater significance than temperature responses is the reduction in aflatoxin production by the addition of DMSO. The increase in aflatoxin production with 5,000 ppm DMSO

TABLE 1. Effect of dimethyl sulfoxide on mycelium and aflatoxin production by *Aspergillus flavus* after 7 days' incubation at three temperatures

ppm DMSO × 1,000	Temp (C)					
	22		28		35	
	Mycelium[a]	Aflatoxin[b]	Mycelium	Aflatoxin	Mycelium	Aflatoxin
0	933	11.5	911	11.0	1,170	13.4
5	978	17.6	885	15.6	1,142	10.4
10	976	12.4	936	10.7	1,173	8.1
25	773	4.8	725	6.4	1,082	2.8
30	787	4.7	760	4.6	949	1.7
35	835	3.7	728	2.7	1,027	0.9
40	678	2.2	739	1.7	957	0.7
45	788	1.6	695	1.1	984	0.3
50	610	0.5	689	0.6	894	0.2

[a] Average mg dry wt of mycelium per flask.
[b] Average mg of aflatoxin per flask.

in the medium occurred only twice in this experiment, after 2 weeks at 35 C and after 4 weeks at 28 C.

The reduction in aflatoxin production by DMSO was verified by thin-layer chromatography. One-μliter portions of extracts from cultures incubated 1 week at 28 C were compared to aflatoxin standards obtained from L. A. Goldblatt, USDA, New Orleans, La. Fluorescent spots corresponding to aflatoxins B_1, B_2, G_1, and G_2 were similar in intensity in standard, control, 5,000 ppm, and 10,000 ppm treatments. Only aflatoxins B_1 and G_1, however, could be detected in filtrates of cultures grown in medium containing 25,000 ppm DMSO; no fluorescence was detected in filtrates from cultures grown in 45,000 ppm or 50,000 ppm DMSO. When 50 μliters of extract were spotted, fluorescence was detectable in the 40,000-ppm and 45,000-ppm DMSO treatment; however, the spots were not typical of aflatoxin standard.

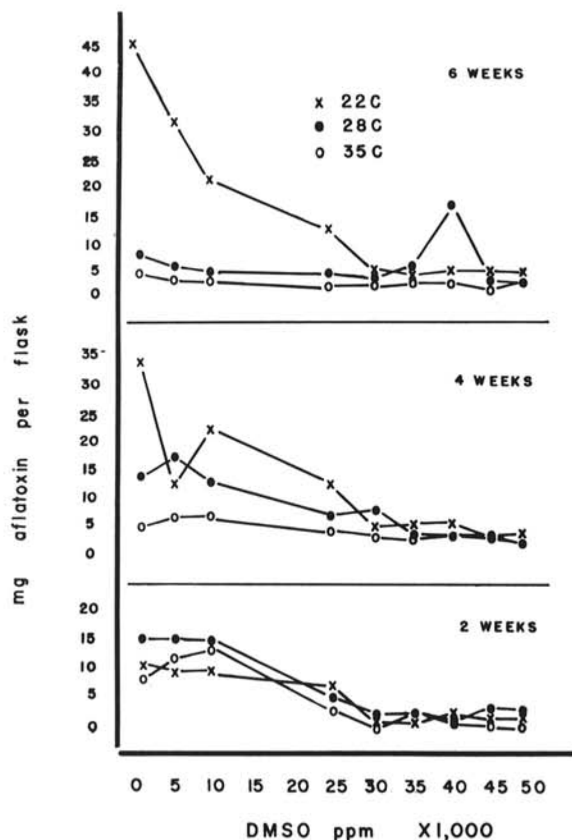

Fig. 1. Influence of dimethyl sulfoxide on aflatoxin concentration produced by *Aspergillus flavus* after 2, 4, and 6 weeks' incubation at three temperatures.

In order to test the interaction of DMSO and aflatoxins, DMSO was added to 7-day-old culture filtrates of *A. flavus* to achieve 50,000 ppm DMSO. DMSO was also added directly to aflatoxin standards in chloroform. After 24 hr, the level of aflatoxin was determined using the MEQ and compared to aflatoxin level in the standard and culture filtrate without DMSO; the levels were the same.

Mycelium produced in medium containing 50.000 ppm DMSO was homogenized in medium with a Virtis homogenizer for 2 min at 45,000 rpm and centrifuged at 12,000 *g* for 30 min, and aflatoxin was extracted from the liquid fraction. Mycelium grown in DMSO-free medium was the control. The ratio of aflatoxin in the culture filtrate to aflatoxin in the mycelium was 10:1 for the control and 23:1 for the 50,000 ppm DMSO treatment.

DISCUSSION.—The means by which DMSO reduces aflatoxin production were not determined except to demonstrate that DMSO does not degrade already produced aflatoxin; however, it appears to affect the rate at which aflatoxin is released from the fungal mycelium. Since DMSO complexes with many metals (10), it may prevent aflatoxin production by making metals in the substrate, such as zinc (9), unavailable for incorporation into the aflatoxin molecule.

Although DMSO is not fungicidal against *A. flavus*, prevention of aflatoxin production in plant products, such as peanuts, might be possible by treatment with DMSO at harvest. DMSO has not been released for use in human food but it has low animal toxicity (14), thus eliminating the problem of harmful residue. Dimethyl sulfoxide might be used in combination with other compounds such as *p*-amino benzoic acid, potas-

sium sulfite, or potassium fluoride also reported to inhibit aflatoxin production (4).

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