APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1980, p. 352-357
0099-2240/80/08-352/06$02.00/0

Vol. 40, No. 2

Inhibition of *Aspergillus* Growth and Aflatoxin Release by Derivatives of Benzoic Acid†

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A study was conducted to determine the effects of *o*-nitrobenzoate, *p*-aminobenzoate, benzocaine (ethyl aminobenzoate), ethyl benzoate, methyl benzoate, salicylic acid (*o*-hydroxybenzoate), *trans*-cinnamic acid (*β*-phenylacrylic acid), *trans*-cinnamaldehyde (3-phenylpropenal), ferulic acid (*p*-hydroxy-3-methoxycinnamic acid), aspirin (*o*-acetoxy benzoic acid), and anthranilic acid (*o*-aminobenzoic acid) upon growth and aflatoxin release in *Aspergillus flavus* NRRL 3145 and *A. parasiticus* NRRL 3240. A chemically defined medium was supplemented with various concentrations of these compounds and inoculated with spores, and the developing cultures were incubated for 4, 6, and 8 days at 27°C in a mechanical shaker. At the beginning of day 8 of incubation, aflatoxins were extracted from cell-free filtrates, separated by thin-layer chromatography, and quantitated by ultraviolet spectrophotometry. The structure of these aromatic compounds appeared to be critically related to their effects on mycelial growth and aflatoxin release. At concentrations of 2.5 and 5.0 mg per 25 ml of medium, methyl benzoate and ethyl benzoate were the most effective in reducing both mycelial growth and aflatoxin release by *A. flavus* and *A. parasiticus*. Inhibition of mycelial growth and aflatoxin release by various concentrations of the above-named aromatic compounds may indicate the possibility of their use as fungicides.

Aflatoxins, produced by certain strains of *Aspergillus flavus* and *A. parasiticus*, have been well documented as producing significant pathological changes in plants and animals (6, 13). Recent evidence has indicated that aflatoxin may also be involved in the etiology of human liver cancer in certain parts of the world (17). The need for protection of foods and feedstuffs against *A. flavus* is universally recognized, and several approaches have been suggested. Applegate and Chipley (2, 3) reported that spore germination of toxigenic strains of *A. flavus* could be inhibited by exposure of the spores to 400 or 600 krad of gamma irradiation. Rao and Harein (14), Schroeder et al. (16), and Yao and Hsieh (22) reported that dichlorvos (dimethyl 2,2-chlorovinyl phosphate) at 20 μg/ml inhibited aflatoxin production in *A. flavus*. Recently, it was shown that aflatoxin production by *A. flavus* in synthetic media could be greatly reduced by benzoic acid or sodium benzoate (20). The reduction was accompanied by the formation of a yellow pigment.

Phenolic compounds are produced by various plants. Among the better known are the derivatives of benzoic acid, such as salicylate, *p*-hydroxybenzoate, and derivatives of cinnamic acid, such as coumaric acid, as well as coumarin, and related compounds (11). Some phenolic acids, such as salicylate, and some flavinoids have been reported as inhibiting plant growth (10).

The role of several metabolic inhibitors and promoters of aflatoxin biosynthesis has been reviewed recently (12). Among the compounds that were reported tested in cultures of *A. parasiticus* were *p*-aminobenzoic, *p*-aminosalicylic, *p*-nitrobenzoic, anthranilic, and salicylic acids. However, the authors stated that these compounds showed large variations in their effects in suspension and growth media (affecting aflatoxin production and fungal biomass, respectively).

Benzoates and their derivatives have been used extensively as antimicrobial agents. Their effectiveness increases as the acidity of the surrounding medium increases, which indicates that the undissociated acid is the effective agent. The mechanism of action of these compounds is not clear. However, it is well documented that their effectiveness increases with an increase in the chain length of the ester group. A study to explore their use as fungicides, especially against the growth of toxigenic cultures such as *Aspergillus*, would be warranted. Therefore, the present study was conducted to determine the effects of these compounds on mycelial growth and

† Ohio Agricultural Research and Development Center Journal Article Series No. 48-78.

release of aflatoxins in synthetic media by toxigenic strains of *A. flavus* and *A. parasiticus*.

MATERIALS AND METHODS

Organisms. *A. flavus* 3145 and *A. parasiticus* (*A. flavus*) 3240, used throughout this investigation, were obtained from the culture collection of the Northern Regional Research Laboratory, Agricultural Research Service, Peoria, Ill. Stock cultures were grown initially on potato dextrose agar slants for 24 h at 25°C. Reference stock cultures were stored at room temperature under sterile mineral oil by the method of Werham (21). Working stock cultures were obtained by subculturing mycelia from mineral oil-covered slants to screw-cap vials (16 by 125 mm), each vial containing a 15-ml potato dextrose agar slant. Cultures used for spore development and subsequent substrate inoculation were obtained from working stock cultures after 14 days of incubation at room temperature. Spore suspensions were prepared by adding sterile distilled water to 14-day-old sporulated cultures, and spores were collected by membrane ultrafiltration. Spores were then washed twice using 50 ml of distilled water and refiltered.

Growth media. The basal medium used was that of Adye and Mateles (1) supplemented with various concentrations of *o*-nitrobenzoate, *p*-aminobenzoate, benzocaine (ethyl aminobenzoate), ethyl benzoate, methyl benzoate, salicylic acid (*o*-hydroxybenzoate), *trans*-cinnamic acid (β-phenylacrylic acid), *trans*-cinnamaldehyde (3-phenylpropenal), ferulic acid (*p*-hydroxy-3-methoxycinnamic acid), aspirin (*o*-acetoxy benzoic acid), and anthranilic acid (*o*-aminobenzoic acid). Unless otherwise specified, the initial pH of the medium was 4.5. The medium was filter sterilized using a filter pore size of 0.45 μm, and 25 ml was dispensed into each of several sterile 125-ml Erlenmeyer flasks. A 1-ml volume of spore suspension containing 10^6 spores per ml was inoculated into each flask. All benzoate derivatives were of reagent grade, and stock solutions (100 mg/ml) were prepared by dissolving these compounds in 1.0 ml of ethanol. Twenty-four hours after inoculation of media with spores, various concentrations of these derivatives were added. Appropriate controls were inoculated with ethanol to determine any effects of this reagent upon growth and toxin release.

Aflatoxin analysis. Mycelial growth was separated from the liquid phase by filtering through 900s laboratory tissue (Kimberly-Clark) into a 250-ml separatory funnel. Chloroform (50 ml) was added to the filtrate, the mixture was vigorously shaken for 60 s, and the procedure was repeated. The chloroform layers were drawn off into a 500-ml round-bottom flask and brought to a concentration of approximately 10 ml in a rotary flash-evaporator (Buchler Instruments Div., Nuclear-Chicago Corp.). The residue was transferred to a glass culture tube (13 by 100 mm) with two 2-ml chloroform washes and evaporated to dryness under nitrogen gas in a micro-N-evaporator (Organomotion Associates).

Aflatoxins were dissolved in 100 μl of chloroform and separated by thin-layer chromatography using plates coated to a thickness of 250 μm with MN-Kieselgel G-HR silica gel (Brinkmann Instruments Inc.) and developed twice with chloroform-acetone (9:1, vol/vol). The spots representing aflatoxins B_1 and G_1 were removed from the plates, dissolved in 2.2 ml of benzene-acetonitrile (98:2, vol/vol), and quantitated separately using a DU-2 spectrophotometer (Beckman Instruments, Inc.) (3). Commercial standards were cochromatographed, and calibration curves were also prepared for spectrophotometric quantitation of each toxin (19).

Preliminary results indicated that the most significant differences between control and treated cultures in both mycelial dry weights ($P < 0.01$) and quantities of aflatoxins in the media ($P < 0.05$) occurred when flasks were removed at the beginning of day 8 of incubation. Therefore, all results have been reported on the basis of this time period. Data for days 4 and 6 of incubation, although significant ($P < 0.05$ only), have not been given in the present study.

RESULTS

Results of the present investigation indicated that both mycelial growth and release of aflatoxins by *A. flavus* and by *A. parasiticus* were affected by several benzoic acid derivatives. Significant concentration-dependent inhibition of both growth and toxin release was evident for selected derivatives. At a concentration of 2.5 mg per 25 ml of medium, *o*-nitrobenzoate (Table 1) and *p*-aminobenzoate stimulated growth of *A. flavus* but reduced the release of aflatoxins. Ethyl aminobenzoate (benzocaine), at an identical concentration, reduced growth but stimulated the release of aflatoxins. A 5-mg amount of either *o*-nitrobenzoate, *p*-aminobenzoate, or ethyl aminobenzoate per 25 ml of medium reduced mycelial growth or the release of aflatoxins, or both. Complete inhibition of both growth and toxin release occurred with inhibitor concentrations of 100, 225, and 20 mg per 25 ml, respectively (data not shown). Ethyl benzoate (2.5 mg per 25 ml) significantly reduced growth and the release of aflatoxins. Release of aflatoxin was completely inhibited by 5 mg per 25 ml of this compound whereas mycelial growth was reduced by 78%. Methyl benzoate (2.5 mg per 25 ml) reduced mycelial growth (61%) and completely inhibited the release of aflatoxins. The highest reduction (90%) of mycelial growth by this compound was achieved at a concentration of 10 mg per 25 ml (data not shown). Salicylic acid (*o*-hydroxybenzoate; 2.5 mg per 25 ml) reduced mycelial growth (36%) and the release of aflatoxin with complete inhibition of both growth and aflatoxin release at a concentration of 20 mg per 25 ml (data not shown). Anthranilic acid, up to a concentration of 10 mg per 25 ml, significantly increased mycelial growth (over 100%) and the release of aflatoxin G_1, whereas at a concentration of 225 mg per 25 ml, mycelial

TABLE 1. *Effects of benzoate derivatives on growth and release of aflatoxins by A. flavus NRRL 3145*

Treatment	Concn (mg per flask)	Mycelial dry wt		Aflatoxins			
		mg per flask[a]	±SD (mg)	μg per flask[a]		±SD (μg)	
				B_1	G_1	B_1	G_1
Control	0	1,000[a]	50	7.0[a]	6.4[a]	0.60	0.10
o-Nitrobenzoate	2.5	1,100[b]	10	5.0[b]	5.0[b]	0.50	0.50
	5.0	900[b]	7	4.4[b]	4.2[b]	0.20	0.60
	10.0	600[b]	10	3.2[b]	3.0[b]	0.10	0.30
p-Aminobenzoate	2.5	1,200[b]	50	6.2[a]	6.1[a]	0.33	0.54
	5.0	1,000[a]	40	5.7[b]	5.0[b]	0.45	0.10
	10.0	600[b]	8	4.8[b]	4.3[b]	0.70	0.55
Benzocaine	2.5	890[b]	32	7.1[a]	7.0[a]	0.61	0.37
	5.0	620[b]	47	4.3[b]	4.0[b]	0.24	0.38
Ethyl benzoate	2.5	400[b]	30	2.3[b]	2.0[b]	0.20	0.19
	5.0	220[b]	21	—[c]	—	—	—
Methyl benzoate	2.5	390[b]	9	—	—	—	—
	5.0	300[b]	27	—	—	—	—
Salicylic acid	2.5	640[b]	37	5.8[b]	5.5[a]	0.32	0.70
	5.0	520[b]	28	—	—	—	—
Trans-cinnamic acid	2.5	880[b]	29	3.6[b]	3.3[b]	0.21	0.24
	5.0	780[b]	22	—	—	—	—
Trans-cinnamaldehyde	2.5	1,000[a]	39	5.5[b]	5.0[b]	0.30	0.41
	5.0	380[b]	22	—	—	—	—
	10.0	300[b]	28	—	—	—	—
Ferulic acid	2.5	830[b]	24	5.2[b]	5.0[b]	0.28	0.26
	5.0	700[b]	38	3.5[b]	3.4[b]	0.18	0.26
Anthranilic acid	2.5	1,200[b]	59	7.2[a]	7.0[b]	0.31	0.33
	5.0	2,100[b]	63	7.5[a]	7.1[b]	0.37	0.30
	10.0	2,200[b]	74	8.1[a]	8.0[b]	0.42	0.39
	25.0	1,000[a]	35	5.4[b]	5.0[b]	0.23	0.21
Aspirin	2.5	960[a]	40	3.4[b]	4.0[b]	0.51	0.49
	5.0	1,000[a]	23	7.0[a]	7.0[a]	0.47	0.41
	10.0	1,100[b]	37	7.0[a]	6.5[a]	0.30	0.50

[a] Average of four experiments; values in the same column which are not followed by the same superscript are significantly different ($P < 0.01$) for mycelial dry weights and for aflatoxin content ($P < 0.05$).

[c] —, None detected.

growth was reduced (75%) and the release of aflatoxins was completely inhibited (data not shown). *trans*-Cinnamic acid (β-phenylacrylic acid; 2.5 mg per 25 ml) significantly reduced mycelial growth and the release of aflatoxins. Aflatoxin release was completely inhibited by 5 mg of this compound per 25 ml and by 5 mg of *trans*-cinnamaldehyde (3-phenylpropenal) per 25 ml. Ferulic acid (*p*-hydroxy-3-methoxycinnamic acid) (2.5 and 5 mg per 25 ml) significantly inhibited mycelial growth and release of aflatoxins. Aspirin (*o*-acetoxy benzoic acid; 2.5 mg per 25 ml) significantly inhibited release of aflatoxins.

Benzoate derivatives also affected mycelial growth and release of aflatoxins by *A. parasiticus* (Table 2). *p*-Aminobenzoate, at a concentration of 10 mg per 25 ml, significantly decreased mycelial growth (10%) and aflatoxin release. Ethyl benzoate (5 mg per 25 ml), methyl benzoate (2.5 and 5 mg per 25 ml), salicylic acid (5 mg per 25 ml), *trans*-cinnamic acid (5 mg per 25 ml), and *trans*-cinnamaldehyde (5 mg per 25 ml) all completely inhibited the release of aflatoxins. Reduction of mycelial growth ranged from 13 to 65% for these compounds. Ferulic acid (5 mg per 25 ml) significantly inhibited aflatoxin release (75%) and reduced mycelial growth (39%). Anthranilic acid, up to a concentration of 10 mg per 25 ml, significantly increased mycelial growth (up to 30%). *o*-Nitrobenzoate decreased mycelial growth and, as with higher concentrations of aspirin, decreased the release of aflatoxins. Benzocaine (5 mg per 25 ml) significantly reduced growth (25%) and release of aflatoxins (50%).

DISCUSSION

Several of the benzoic acid derivatives used in the present study reduced mycelial growth or aflatoxin release, or both. This suggested that the structure of these compounds might determine their effectiveness as fungicidal agents. It is also of interest to note that, in both *A. flavus* and *A. parasiticus*, control of mycelial growth

was apparently independent of aflatoxin release, i.e., a 50% reduction of mycelial growth did not necessarily result in a 50% reduction in the amount of aflatoxin released.

Davis and Diener (7) reported that each of several concentrations of *p*-aminobenzoic acid and anthranilic acid inhibited aflatoxin production by *A. parasiticus* Speare var. *globosum* in yeast extract-sucrose medium. Higher concentrations of each of these compounds inhibited growth whereas lower concentrations resulted in a stimulation of growth. Similar results were observed in the present study.

Gupta et al. (8) reported that salicyclic acid inhibited aflatoxin synthesis by *A. parasiticus* NRRL 3240 by possibly reducing the synthesis of pantothenic acid. They also postulated that the uncoupling of oxidative phosphorylation with a subsequent decrease in adenosine 5'-triphosphate production could occur from the addition of salicyclic acid to media. Cheshire and Park (5) recently reported that porcine lactate dehydrogenase could be inhibited by salicylate. These authors concluded that salicylate competed with the adenosine monophosphate moiety of nicotinamide adenine dinucleotide in this enzyme. The inhibition of plant malate and isocitrate dehydrogenases by salicyclic acid has also been reported (9). Effective inhibition of aflatoxin release by salicyclic acid was demonstrated in the present study. Thus, this compound is apparently inhibitory to several eucaryotic enzyme systems.

Recently, a review was published in which the role of several metabolic inhibitors and promoters of aflatoxin biosynthesis was discussed (12). This review stated that the addition of *p*-aminobenzoic acid (1 mM) to cultures of *A. parasiticus* had no effect on aflatoxin B and G production. Higher concentrations (10 mM) inhibited the formation of these toxins by 74%. Addition of *p*-aminosalicyclic acid (1 mM) stimulated aflatoxin B and G production by 26 and 8%, respectively. Higher concentrations (10 mM)

TABLE 2. *Effects of benzoate derivatives on growth and release of aflatoxins by A. parasiticus NRRL 3240*

Treatment	Concn (mg per flask)	Mycelial dry wt		Aflatoxins			
		mg per flask[a]	±SD (mg)	μg per flask[a]		±SD (μg)	
				B_1	G_1	B_1	G_1
Control	0	1,000[a]	35	8.2[a]	8.0[a]	0.41	0.52
o-Nitrobenzoate	2.5	900[b]	20	7.8[a]	7.7[a]	0.26	0.15
	5.0	900[b]	22	7.0[b]	7.0[a]	0.30	0.23
	10.0	880[b]	19	6.6[b]	6.5[b]	0.19	0.24
p-Aminobenzoate	2.5	1,000[a]	38	6.5[b]	6.1[b]	0.20	0.31
	5.0	1,100[b]	31	7.3[a]	7.1[a]	0.32	0.45
	10.0	920[b]	40	6.0[b]	5.8[b]	0.37	0.26
Benzocaine	2.5	890[b]	21	6.0[b]	6.1[b]	0.22	0.29
	5.0	750[b]	23	4.1[b]	3.9[b]	0.30	0.25
Ethyl benzoate	2.5	660[b]	15	4.0[b]	3.0[b]	0.35	0.28
	5.0	380[b]	12	—[c]	—	—	—
Methyl benzoate	2.5	510[b]	20	—	—	—	—
	5.0	350[b]	18	—	—	—	—
Salicylic acid	2.5	950[a]	31	5.4[b]	5.0[b]	0.25	0.17
	5.0	870[b]	23	—	—	—	—
Trans-cinnamic acid	2.5	660[b]	25	5.0[b]	3.5[b]	0.29	0.34
	5.0	480[b]	19	—	—	—	—
Trans-cinnamaldehyde	2.5	1,000[a]	22	4.5[b]	4.0[b]	0.38	0.21
	5.0	360[b]	31	—	—	—	—
	10.0	360[b]	26	—	—	—	—
Ferulic acid	2.5	730[b]	32	4.7[b]	4.6[b]	0.40	0.36
	5.0	610[b]	17	2.0[b]	1.8[b]	0.31	0.22
Anthranilic acid	2.5	1,200[b]	34	8.0[a]	7.8[a]	0.42	0.32
	5.0	1,300[b]	39	9.5[a]	8.0[a]	0.56	0.29
	10.0	1,100[b]	41	8.2[a]	7.8[a]	0.33	0.41
	25.0	970[a]	27	6.5[b]	6.3[b]	0.37	0.33
Aspirin	2.5	980[a]	27	8.0[a]	7.6[a]	0.41	0.30
	5.0	980[a]	16	7.8[a]	7.5[a]	0.20	0.26
	10.0	1,000[a]	24	7.0[b]	6.0[b]	0.36	0.35

[a] Average of four experiments; values in the same column which are not followed by the same superscript are significantly different ($P < 0.01$) for mycelial dry weights and for aflatoxin content ($P < 0.05$).

[c] —, None detected.

inhibited toxin formation by 88%. In a similar manner, *p*-nitrobenzoic acid at low concentrations (1 mM) stimulated toxin formation by 20%, whereas higher levels (10 mM) completely inhibited formation. Anthranilic acid (10 mM) inhibited toxin formation by 85%, whereas salicyclic acid (1 mM) inhibited formation by 38%. In general, there was no significant inhibition of fungal growth by the addition of the above compounds. Differences in the results reported in the above studies with those reported in the present study may possibly be related to both differences in concentrations of the compounds studied and differences in media and methods of incubation. Furthermore, the authors (12) in citing the above results failed to differentiate between production or formation of toxins within the fungal biomass and release of toxins into media.

It has also been reported that the addition of cinnamon oil, clove oil, cinnamic aldehyde, and eugenol inhibited both growth and aflatoxin production by *A. parasiticus* in yeast extract-sucrose broth (4). The authors concluded that the effect of these substances was due to inhibition of growth rather than of toxin production. However, in the present study, release of aflatoxins into media appeared to be more sensitive to inhibition by derivatives of benzoate than was the growth of fungi.

In the present study, the compounds which were most effective in reducing both mycelial growth and aflatoxin release by *A. flavus* were methyl benzoate, ethyl benzoate, *trans*-cinnamaldehyde, salicylic acid, and *trans*-cinnamic acid. Compounds which were most effective against *A. parasiticus* were methyl benzoate, ethyl benzoate, *trans*-cinnamaldehyde, *trans*-cinnamic acid, and salicylic acid. On the other hand, anthranilic acid stimulated mycelial growth of both *A. flavus* and *A. parasiticus*.

It is also of interest to note that the reduction of aflatoxin release by these compounds was often accompanied by the appearance of a yellow pigment in the culture media. Although in the present study no analyses were conducted to identify this pigment, a similar phenomenon was noted and the pigment was partially characterized as closely related to an acetyl derivative of a versiconal-type compound when cultures of *A. flavus* were incubated with benzoic acid or sodium benzoate (20).

The successful inhibition of both mycelial growth and release of aflatoxins by some of the aromatic compounds used in the present study indicates the possibility of their use as plant fungicides, especially against the growth of toxigenic cultures of *Aspergillus*. However, further investigation should be undertaken to determine the possible toxic effects of any aromatic residues.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Cliff B. Bennett who performed the statistical analysis for this study.

LITERATURE CITED

1. **Adye, J. C., and R. I. Mateles.** 1964. Incorporation of labelled compounds into aflatoxins. Biochem. Biophys. Acta **86**:418–420.
2. **Applegate, K. L., and J. R. Chipley.** 1973. Effects of ^{60}Co gamma irradiation on aflatoxin B_1 and B_2 production by *Aspergillus flavus*. Mycologia **66**:436–445.
3. **Applegate, K. L., and J. R. Chipley.** 1974. Daily variation in the production of aflatoxins by *Aspergillus flavus* NRRL-3145 following exposure to ^{60}Co irradiation. J. Appl. Bacteriol. **37**:359–372.
4. **Bullerman, L. B., F. Y. Lieu, and S. A. Seier.** 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils. Cinnamic aldehyde and eugenol. J. Food Sci. **42**:1107–1109.
5. **Cheshire, R. M., and M. V. Park.** 1977. The inhibition of lactate dehydrogenase by salicylate. Int. J. Biochem. **8**:637–643.
6. **Clerk, G. C., and M. Caurie.** 1968. Biochemical changes caused by some *Aspergillus flavus* species in root tuber cassava (*Manihot esculenta crantz*). Top. Sci. **10**:149–154.
7. **Davis, N. D., and U. L. Diener.** 1967. Inhibition of aflatoxin biosynthesis by *p*-amino benzoic acid, potassium sulfite, and potassium fluoride. Appl. Microbiol. **15**:1517–1518.
8. **Gupta, S. R., H. R. Prasanna, L. Viswanathan, and T. A. Venkitasubramanian.** 1975. Synthesis of aflatoxins by the non-growing mycelia of *Aspergillus parasiticus* and the effect of inhibitors. J. Gen. Microbiol. **91**:417–420.
9. **Hines, W. J. W., and M. J. H. Smith.** 1964. Inhibition of dehydrogenases by salicylate. Nature (London) **201**: 192.
10. **Kefeli, V. L., and C. S. Kadyrov.** 1971. Natural growth inhibitors: their chemical and physiological properties. Annu. Rev. Plant Physiol. **22**:185–196.
11. **Leopold, A. C.** 1964. Plant growth and development. McGraw-Hill Book Co., New York.
12. **Maggon, K. K., S. K. Gupta, and T. A. Venkitasubramanian.** 1977. Biosynthesis of aflatoxins. Bacteriol. Rev. **41**:822–855.
13. **Muller, R. D., C. W. Carlson, G. Semeniuk, and G. S. Harshfield.** 1970. The response of chicks, ducklings, goslings, pheasants, and poults to graded levels of aflatoxins. Poul. Sci. **49**:1346–1350.
14. **Rao, H. R. G., and P. K. Harein.** 1972. Dichlorvos as an inhibitor of aflatoxin production on wheat, corn, rice, and peanuts. J. Econ. Entomol. **65**:988–989.
15. **Rothe, G. M.** 1976. Inhibition of plant dehydrogenase isoenzymes by benzoic acid and cinnamic acid derivatives. Z. Pflanzephysiol. Bd. **79**:384–391.
16. **Schroeder, H. W., R. J. Cole, R. D. Gridsby, and H. Hein, Jr.** 1974. Inhibition of aflatoxin production and tentative identification of an aflatoxin intermediate "versiconal acetate" from treatment with dichlorvos. Appl. Microbiol. **27**:394–399.
17. **Shank, R. C., G. N. Wogan, and J. B. Gibson.** 1972. Dietary aflatoxins and human liver cancer. 1. Toxigenic moulds in foodstuffs of tropical South-East Asia. Food Cosmet. Toxicol. **10**:51–56.
18. **Steel, G. P., and J. H. Torrie.** 1960. Principles and

procedures of statistics. McGraw-Hill Book Co., New York.

19. **Uraih, N., and J. R. Chipley.** 1976. Effects of various acids and salts on growth and aflatoxin production by *Aspergillus flavus*. Microbios **17**:51-59.
20. **Uraih, N., T. R. Cassity, and J. R. Chipley.** 1977. Partial characterization of the mode of action of benzoic acid on aflatoxin biosynthesis. Can. J. Microbiol. **23**: 1580-1584.
21. **Werham, C. C.** 1946. Mineral oil as a fungus culture preservative. Mycologia **38**:691-693.
22. **Yao, R. C., and D. P. H. Hsieh.** 1974. Step of dichlorvos inhibition in the pathway of aflatoxin biosynthesis. Appl. Microbiol. **28**:52-57.