Effects of Dichlorvos on Ochratoxin Production by *Aspergillus ochraceus*

The organophosphate insecticide, dichlorvos, introduced at a level of 1 mg/100 ml of yeast extract-sucrose medium, reduced the production of ochratoxin A and B by ¼ to ½. At a concentration of 10–30 mg/100 ml of medium, dichlorvos inhibited from ½ to ⅘ of ochratoxin A production and from ½ to 9/10 of ochratoxin B production. From 9 to 21% of *Aspergillus ochraceus* growth was retarded at 10–30 mg/100 ml of yeast extract-sucrose medium. Dichlorvos had a similar inhibitory effect on mold on corn.

Aspergillus ochraceus has been isolated from various agricultural commodities (Christensen and Gordon, 1948; Scott, 1965; Van der Merwe *et al.*, 1965). Three of five isolates of *A. ochraceus* were found to be toxic to mice, rats, and ducklings (Scott, 1965). The toxic compound was later identified as ochratoxin A (Van der Merwe *et al.*, 1965). The toxicity of ochratoxin A is well established; this metabolite is about ten times as toxic as ochratoxin B to day-old chicks (Peckham *et al.*, 1971). Natural occurrence of ochratoxin A in moldy corn, wheat, and other agricultural products has been reported (Shotwell *et al.*, 1969; Scott *et al.*, 1972).

The organophosphate insecticide dichlorvos (dimethyl 2,2-dichlorovinyl phosphate) has shown promise as a protective agent for harvested grain from infestation by various species of stored product insects (Strong and Sbur, 1961, 1964; Kirkpatrick *et al.*, 1968). It was recently shown that dichlorvos strongly inhibited aflatoxin production by *Aspergillus flavus* (Rao and Harein, 1972; Hsieh, 1973) and zearalenone production by *Fusarium roseum* Graminearum (Wolf *et al.*, 1972; Wolf and Mirocha, 1973).

The present investigation was undertaken to determine growth and toxin production by *A. ochraceus* when dichlorvos was introduced into laboratory medium or natural substrate.

MATERIALS AND METHODS

Aspergillus ochraceus strain UGa no. 40, isolated from peanuts, and *A. ochraceus* NRRL 3174, a known ochratoxin producing strain, were used in this study. After inoculation onto Czapek-Dox agar slants, cultures were incubated for 2 weeks at 27° and then stored at 4°. For culturing on yeast extract-sucrose medium (2% yeast extract, 20% sucrose), various amounts of dichlorvos (Shell Chemical Co., San Ramon, Calif.) were added aseptically to the autoclaved media. For culturing on corn, 30 ml of sterilized distilled water and dichlorvos were added to the autoclaved corn. Spores (10^6) of each strain of *A. ochraceus* were added and incubated at 27° for 14 days. For measuring the dry weight of mycelia from the yeast extract-sucrose medium, after autoclaving, mycelial pads were separated from the culture filtrate, rinsed with distilled water, and dried in an oven for 3 hr.

After steaming the culture to facilitate extraction of ochratoxins, the contents were transferred to 1-qt Mason jars and extracted by homogenizing three times with 50 ml of chloroform. The extracts were combined, concentrated in a flash evaporator, diluted to 5 ml with chloroform, and separated by thin-layer chromatography on Adsorbosil-1 (Applied Science Laboratories, State College, Pa.) with toluene-ethyl acetate-formic acid (5:4:1, v/v/v) as a developing solvent (Eppley, 1968). Upon treatment of the chromatogram with ammonia, the presence of ochratoxin was detected by the appearance of a blue fluorescence when viewed under ultraviolet light. A Photovolt fluorodensitometer (Photovolt Corporation, New York, N. Y.) was used to compare the intensity of fluorescence of the samples with that of standards furnished by the Bureau of Food and Drug Sanitation, Food and Drug Administration, Washington, D. C.

RESULTS AND DISCUSSION

As shown in Table I, dichlorvos at 0.1 mg/100 ml of medium had no effect on growth or ochratoxin production by either strain of *A. ochraceus*. At 1 mg/100 ml, growth was not affected, but ochratoxin A production was inhibited by 48% (no. 40) and 27% (NRRL 3174). Growth of strain no. 40 was retarded by 9–20% in the presence of dichlorvos at 10–30 mg/100 ml of medium. Production of ochratoxin A was inhibited by 72–79% and that of ochratoxin B by 62–89%. For strain NRRL 3174, growths were retarded by 11–18% when dichlorvos was added at a level of 10–30 mg/100 ml of broth, while ochratoxin A production was reduced by 50–74% and ochratoxin B by 49–78% of control.

Dichlorvos also inhibited ochratoxin production by *A. ochraceus* on corn (Table II). At 0.1 mg/100 g, ochratoxin production by either strain apparently was not inhibited. At 1–30 mg/100 g of corn, from ¼ to ¾ of the ochratoxin A ordinarily produced by strain no. 40 was inhibited, while that of ochratoxin B was reduced by ¼ to ⅚. At the same dichlorvos dosage, ochratoxin A production by strain NRRL 3174 was reduced by about ¼ to ⅞ and ochratoxin B by ½ to 9/10.

It is apparent that dichlorvos not only impaired aflatoxin formation by *A. flavus* and zearalenone production by *Fusarium roseum* Graminearum as reported by others (Rao and Harein, 1972; Hsieh, 1973; Wolf *et al.*, 1972; Wolf and Mirocha, 1973) but also inhibited ochratoxin production by *A. ochraceus*. However, it seems that higher concentrations of the insecticide are necessary (10–300 ppm) for inhibition of ochratoxin production than for aflatoxin (5–20 ppm) and zearalenone (10 ppm) production. At relatively high concentrations (100–300 ppm) both growth and toxin production were inhibited. Dichlorvos at these concentrations seems to have a fungistatic effect on *A. ochraceus*. The mechanism of inhibition of ochratoxin production by dichlorvos is unknown, but reduced aflatoxin production has been attributed to the inhibition of acetate incorporation (Hsieh, 1973).

Dichlorvos is a widely used insecticide whose residues have been detected in stored grain (Rowlands, 1971). It is possible that residual dichlorvos may play a role in the inhibition of these mycotoxins in a natural environment if these grains happen to be infected. Also, dichlorvos may be used primarily to control mycotoxin production in agricultural commodities if this chemical does have broad antimycotoxic effect.

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Table I. Effects of Dichlorovos on Growth and Ochratoxin Production in Yeast Extract–Sucrose Medium

Strain	Dichlorvos, mg/100 ml of broth	Dry mycelia, g/100 ml of broth	% control	Ochratoxin A, mg/100 ml of broth	% control	Ochratoxin B, mg/100 ml of broth	% control
UGa no. 40	0	3.54 ± 0.13[a]		3.77 ± 0.24		2.59 ± 0.09	
	0.1	3.49 ± 0.10	99	3.69 ± 0.29	98	2.64 ± 0.11	102
	1	3.57 ± 0.16	101	2.68 ± 0.20	71	1.35 ± 0.10	52
	10	3.24 ± 0.09	91	1.05 ± 0.08	28	0.99 ± 0.07	38
	30	2.80 ± 0.10	80	0.81 ± 0.11	21	0.29 ± 0.05	11
NRRL 3174	0	2.86 ± 0.12		5.15 ± 0.22		1.72 ± 0.07	
	0.1	2.91 ± 0.14	102	5.19 ± 0.28	101	1.68 ± 0.09	98
	1	2.78 ± 0.10	97	4.05 ± 0.19	79	1.25 ± 0.09	73
	10	2.54 ± 0.08	89	2.55 ± 0.26	50	0.88 ± 0.07	51
	30	2.34 ± 0.11	82	1.34 ± 0.16	26	0.38 ± 0.05	22

[a] Average of three replications.

Table II. Effects of Dichlorvos on Ochratoxin Production on Corn

Strain	Dichlorvos, mg/100 g of corn	Ochratoxin A, mg/100 g of corn	% control	Ochratoxin B, mg/100 g of corn	% control
UGa no. 40	0	3.65 ± 0.30[a]		1.84 ± 0.17	
	0.1	3.77 ± 0.24	103	1.77 ± 0.11	96
	1	2.88 ± 0.28	79	1.37 ± 0.09	74
	10	1.63 ± 0.19	45	0.84 ± 0.09	46
	30	0.94 ± 0.08	26	0.32 ± 0.05	17
NRRL 3174	0	4.86 ± 0.35		2.33 ± 0.19	
	0.1	4.69 ± 0.31	97	2.27 ± 0.21	97
	1	3.68 ± 0.27	76	1.15 ± 0.11	49
	10	2.83 ± 0.19	58	0.63 ± 0.08	27
	30	0.67 ± 0.06	14	0.19 ± 0.03	8

[a] Average of three replications.

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Use of a Simplified Method for Lysine by Gas Chromatography

Gas chromatography with a 12-ft column, higher internal standard, and lower temperature and carrier gas rate improved accuracy and precision of lysine determination in 50% longer time than an earlier simplified method

In 1972 we described a short method (Zscheile and Brannaman, 1972) for quantitative determination of lysine in crude acid hydrolysates of wheat and rice seeds. Derivatization was simple and the gas chromatography brief (13 min). Our subsequent use of this method (Ruckman *et al.*, 1973) led to further evaluation of factors affecting reproducibility and to the allowance of more time for chromatography when brevity is not essential.

Following our custom of adding lysine standard [step 5 (Zscheile and Brannaman, 1972) (all steps referred to in this communication are from this reference)] to one sample of each day's run (12–24 samples) and chromatographing with and without lysine standard on both columns, used alternately, we noted occasional fairly large calibration differences between columns. During 17 runs on wheat with 2 sets of columns extending over 1 year, these