Inhibition of Aflatoxin and Zearalenone Biosynthesis with Dichlorvos[1]

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There is considerable research focused on the hazards of chemicals. One of the current concerns is the significant increase in the number of environmental toxicants discovered through multidisciplinary programs (GOLDBLATT 1969). For example, we now more fully recognize toxic metabolites produced by fungi as relatively new chemical hazards to public health. In a recent review on the role of fungal metabolites in carcinogenesis, the two groups implicated as hepatotropic and myelotropic were metabolites of Aspergillus sp. called aflatoxins and some metabolites of Fusarium sp. (ALEKSANDROWICZ et al. 1970).

In terms of dosage, aflatoxins are the most potent of all the known chemical carcinogens. Aflatoxins have also been implicated as one of the causative agents of childhood cirrhosis (AMLA et al. 1970,1971) and autopsies of children with acute encephalopathy and fatty-degeneration of viscera revealed the presence of aflatoxins in various tissues (SHANK et al. 1971).

Certain strains of Fusarium roseum var. graminearum Snyder and Hansen also produce toxic metabolites. Some of these metabolites are known to produce deleterious effects when eaten by swine. This involves the development of swollen, edematous vulva in females, shrunken testes in young males, enlarged mammary glands in the young of both sexes and possibly abortion in pregnant gilts or sows (STOB et al. 1962). The estrogenic metabolite causing these symptoms is called Zearalenone.

DOLLEAR (1969) and GOLDBLATT (1971) reviewed the literature on the control, prevention and detoxification of aflatoxins. Studies in India indicated that the fumigants ethylene oxide, methyl bromide, sulfur dioxide and mixtures of ethylene dibromide and methyl bromide inhibited mold proliferation (NARASIMHAN and RANGASWAMI 1968); RAGHUNATHAN et al. 1969). Data on the effects of the organophosphate insecticide dichlorvos (2,2-dichlorovinyl dimethyl phosphate) on the inhibition of aflatoxin by A. flavus on various substrates was reported by RAO and HAREIN (1972).

The present paper describes the effectiveness of dichlorvos for preventing the biosynthesis of both aflatoxin and Zearalenone in grain.

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MATERIALS AND METHODS

Aspergillus flavus NRRL-2999 and Fusarium roseum var. graminearum were cultured on autoclaved wheat and on a wheat-rice mixture, respectively. The moisture of these substrates was adjusted to 20± 0.2% prior to use. They were autoclaved 30 min at 120°C., shaken to prevent caking and after a 24 hr period, reautoclaved 60 min. Dichlorvos was diluted with water so that a residue of 20 ppm would be applied to the substrates using 1 ml of the final formulation. This 1 ml dichlorvos formulation was applied to the sides of each 3.79 liter jar above the level of the substrate. Immediately after application, each jar was closed with a metal lid and rotated 15 min to distribute the dichlorvos evenly throughout the substrate. Both treated and untreated control substrates were transferred to separate Erlenmeyer flasks in lots of 100 g each. These substrates were then inoculated with 0.1 g of presoaked soil cultures of A. flavus and F. roseum var. graminearum. Substrates containing A. Flavus spores were incubated 7 days at 27°C and 70% RH and those containing F. roseum var. graminearum for 6 weeks at the above mentioned conditions followed by 8 weeks at 10°C. Aflatoxins were extracted and purified by the method of PONS et al. (1970) and quantitated by using the molar extinction at the absorption maximum of 363 nm. Quantitative estimates of Zearalenone were determined according to MIROCHA et al. (1969).

RESULTS AND DISCUSSIONS

Results on the effect of dichlorvos on the biosynthesis of these toxic metabolites are presented below:

TABLE 1

Concentration of Aflatoxin and Zearalenone on the substrates containing 0 and 20 ppm of dichlorvos

Concentration of dichlorvos (ppm) on the substrate	Concentration of metabolites (ppm)	
	Aflatoxin	Zearalenone
0	79	128
0	94	144
0	112	176
20	0	0
20	0	0
20	0	0

Dichlorvos at 20 ppm prevented the production of aflatoxin as well as Zearalenone while the untreated substrates had an average of 95 ppm aflatoxins and 149 ppm Zearalenone. Nevertheless

the vegetative growth of the fungi appeared normal. Also research by RAO and HAREIN (1972) indicated that, although dichlorvos acts as in inhibitor of the biosynthesis of aflatoxin on various substrates, once the synthesis takes place, it does not reduce the concentration of aflatoxin present.

Both aflatoxins and Zearalenone are grouped as nonaketides under the general class polyketides (TURNER 1971). To inhibit the biosynthesis of a wide variety of fungal toxins, the inhibitor should act on some key enzymes. However, little is known about the various enzymatic processes involved in the biosynthesis of polyketides. Available information is usually limited to the precursors involved.

One of the most common intermediates in the synthesis of secondary metabolites of fungi, is acetyl coenzyme-A. (TURNER, 1971). Recent studies suggest that acetate is the precursor for the synthesis of aflatoxins (BIOLLAZ et al. 1968,1970; HOLKER and MULHEIRN 1968). Acetate also seems to be the common precursor in the biosynthesis of resorcylic acid lactones, radicicol (MIRRINGTON et al. 1964; McCAPRA et al. 1965) and Zearalenone. Dichlorvos inhibited the incorporation of labeled acetate onto Zearalenone by 50% compared to the values in untreated F. roseum cultures (LIEBERMAN et al. 1971).

Since dichlorvos inhibits the biosynthesis of these two different fungal metabolites, which have different structural and pharmacological properties, it would be interesting to investigate whether or not dichlorvos inhibits the synthesis of other toxic fungal metabolites. It may interfere with the acetate-malonate pathway, which seems to be quite a common route in the biosynthesis of secondary metabolites of fungi.

REFERENCES:

ALEKSANDROWICZ, J., M. CZACHOR., A. SCHIFFER, and B. SMYK.: Haemat Latina 8, 115 (1970).
AMLA, I., K. SHYAMALA., V. SREENIVASAMURTHY., A. P. JAYARAJ., and H.A.B. PARPIA: Indian Pediatrics 7, 262 (1970).
AMLA, I., C. S. KAMALA., G. S. GOPALAKRISHNA., A. PAULJAYARAJ., V. SREENIVASAMURTHY., and H.A.B. PARPIA: Amer. J. Clin. Nutr. 24, 609 (1971).
BIOLLAZ, M., G. BUCHI., and G. MILNE,: J. Amer. Chem. Soc. 90, 5017 (1968).
BIOLLAZ, M., G. BUCHI., and G. MILNE.: J. Amer. Chem. Soc. 92, 1035 (1970).
DOLLEAR, F. G.: In.Aflatoxin. Scientific background, control and implications (L. A. GOLDBLATT, ed.) pp. 359-91. Academic Press. New York, 1969.
GOLDBLATT, L. A.: J. Amer. Chem. Soc. 48, 605 (1971).
GOLDBLATT, L. A.: In. Aflatoxin: Scientific background, control and implications. (L. A. GOLDBLATT, ed.) pp. 1-11, Academic Press, New York, 1969.
HOLKER, J. S. E., and L. J. MULHEIRN.: Chem. Commun. 1576 (1968).

LIEBERMAN, J. R., J. C. WOLF., H. R. G. RAO., and P. K. HAREIN.: Sci. J. Ser. Minn. Agri. Expt. Stn. No. 7599 (1971).
MIROCHA, C. J., C. M. CHRISTENSEN., and G. H. NELSON.: Apl. Microbiol. 17, 482 (1969).
McCAPRA, F., A. I. SCOTT., P. DELMOTTE., and J. DELMOTTE-PLAQUEE.: Tetrahedron Letters No. 15, 869 (1965).
MIRRINGTON, R. N. E. RITCHIE., C. W. SHOPPEE., W. C. TAYLOR and S. STERNHELL.: Tetrahedron Letters No. 7, 365 (1964).
NARASIMHAN, K. S. and G. RANGASWAMI.: Indian Phytopath., Soc. Bull. 4, 57 (1968).
PONS, W. A., A. F. CUCULLU., and L. S. LEE.: Proc. International Congr. Fd. Sci. Tech. Washington, D. C., p. 705 (1970).
RAGHUNATHAN, A. N., M. MUTHU., and S. K. MAJUMDER.: J. Stored Prod. Res. 5, 389 (1969).
RAO, H. R. GUNDU., and P. K. HAREIN,: J. Econ. Entomol. 65, 988 (1972).
SHANK, R. C., C. H. BOURGEOIS., N. KESCHAMRAS, and P. CHANDAVIMOL.: Fd. Cosmet. Toxicol. 9, 501 (1971).
STOB, M., R. S. BALDWIN., J. Tuite., F. N. Andrews., and K. G. GILLETTE. Nature 196: 1318 (1962).
TURNER, W. B.: Fungal Metabolites Academic Press, New York, 1971.