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Effects of Fenamiphos, Carbofuran, and Aldicarb on Zearalenone Production by Toxigenic *Fusarium* spp. Contaminating Roots and Fruits of Tomato

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Mycotoxins are compounds produced by fungi and contaminate human food and animal feeds. Mycotoxins produced by Fusarium spp. in agricultural commodities cause mycotoxicoses in animals and human beings, such as estrogenic syndromes in farm animals, alimentray toxic aleukia, akakbitoxicoses and scabby grain toxicosis in humans, (Mirocha et al., 1977; Bottalico et al.,1980). The toxins implicated in these mycotoxicoses have been identified as zearalenone and trichothecenes, (Bottalico et al; 1983). Zearalenone is an estrogenic compound which is produced as a secondary metabolite by various species of Fusarium spp. (Bottalico et al. 1983). This compound has been implicated in causing hyperestrogenism, abnormal estrus, infertility, stillbirth, small litters and fetal absorption when ingested by swine (Smith,1980). Some Fusarium metabolites are also phytotoxic and play a part in the development of plant diseases (Bottalico et al., 1980). Berisford and Ayres (1976) found that production of zearalenone was completely inhibited in culture by the insecticide naled. Pesticides also inhibited fungal growth and zearalenone production by Fusarium roseum var. graminearum.(Draughon and Churchville, 1985).

This paper deals with the effect of fenamiphos, carbofuran and aldicarb nematicides in soil on the occurrence of toxigenic Fusarium isolates naturally contaminating roots and tomato fruits and zearalenone production.

MATERIALS AND METHODS

Soil nurseries were treated with the nematicide fenamiphos, carbofuran and aldicarb at planting time with 20 kg/feddan (three plots of 1/200 feddan for each nematicide (1 feddan= 4200 m2)) At harvest, 15 samples each of soil ,tomato roots and tomato fruits were collected and examined for the presence of Fusarium spp. Ten grams of each sample were mixed with 20 ml sterile potassium phosphate monobasic buffer, pH 7 saline and homogenized for 2 minutes using a bench blender. After shaking vigorously, one ml of each dilution was spread on the surface of Sabouraud's dextrose agar meduim of three replicate dishes. Plates were incubated at 25°C for 5-7 days. Representative Fusarium spp.colonies from each plate were sampled and were identified according to Booth's nomenclature (1971). These were maintained on Sabouraud's dextrose agar at 4°C to screen for Fusarium zearalenone-producing strains. A total of 75 random selected isolates of Fusarium spp. were grown in 500 ml Erlenmeyer flasks containing 100 ml of potato-dextrose broth (Difco Laboratory, Detro", Michigan, USA)). The inoculated culture media were kept at room temperature (28±2°C) for 7 days and at 12°C for an additional 21 days. After incubation, cultures were filtered under vaccum using four layers of cheese cloth on a Buchner funnel. Zearalenone was extracted by the procedures of Bottalico et al. (1983). Zearalenone was quantified by direct comparison of the spots of sample extracts with three dilutions of standard mycotoxin solution (10 ,20 and 50 μ g/l).

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RESULTS AND DISCUSSION

The production of zearalenone by Fusarium species isolated from soils, roots and fruits of tomato plants at harvesting is reported in Table 1. A total of 67 strains of Fusarium belonging to 7 species were isolated. The percentage of isolates able to produce zearalenone were 34.5, 38.0 and 47.0 %, respectively for soils, roots and fruits. All strains of F. culmorum (4 strains), F graminearum (11 strains) and F. oxysporum (11 strains) were the main zearalenone producers. Fusarium species are widely distributed in cultivated soils and were reported to invade plants before harvesting and producing mycotoxins (Pitt and Hocking, 1983 and Manka et al. 1985). Through our study (Table 1), the highest average of zearalenone was produced the toxigenic species isolated from soils, roots and fruits, F.culmorum (670, 100 and 0 ppm), F.graminearum (800,500 and 570 ppm) and F.oxysporum (400, 300 and 265 ppm) in liquid medium at 27 °C. These observations agree with Mirocha et al (1977), Smith (1980) and Bottalico et al (1983).

Recently, particular attention has been paid in many countries to mycotoxins produced by Fusarium, particularly to zearalenone. These secondary metabolities show strong zootoxic activity (Mirocha et al..1977 and Jacobellis and Bottalico 1981). Many field outbreak toxicoses as well as chromic diseases of livestock have been reported (Booth, 1983).

The production of zearalenone by Fusarium spp. mainly isolated from roots and fruits of tomato plants at harvesting in soils treated with several nematicides is reported in Table 2. The organophosphate fenamiphos and two carbamates carbofuran and aldicarb reduced the occurrence of toxigenic Fusarium strains naturally contaminating roots by 6, 11 and 0% respectively and tomato fruits by 21,15 and 8% respectively as compared with controls . The application of fenamiphos revealed that zearalenone was produced only by F. graminearum (one strain) isolated from each of root and tomato fruit samples as well as by F.oxysporum (2 strains) isolated from highly decayed tomato fruits. On the other hand, the application of carbofuran revealed that F.oxysporum (one strain) isolated from roots as well as F. graminearum (one isolate) recovered from moderate decayed tomato fruits produced zearalenone. On the application of aldicarb, we did not detect zearalenone producing Fusarium spp. from either roots or tomato fruits. The list of mycotoxins produced by Fusaria is now very long and exceeds 60 compounds. Marre (1980) and Yoder (1980) reported that Fusarium mycotoxins (zearalenone and deoxynivalenols or vomitoxin) can play a part in crops diseases because of their phytotoxic activity. Their zootoxic activity may be dangerous becauce of their acumulation in fruits when maturing fruits are infected by Fusaria. On the other hand, Chelkowski et al; (1984) observed formation of zearalenone by pathogenic isolates of F.culmorum These observations are in agreement with our data, that all strains of F.graminearum and F.oxysporum which produced high concentrations of zearalenone were isolated from highly decayed tomato fruits. Our present results indicate that all nematicides studied are inhibitors of either fungal growth or zearalenone biosynthesis. Draughan & Churchville (1985) showed that . toxin production is usually more sensitive than growth to inhibitors, since metabolites for synthesis of zearalenone are produced during fungal growth. Small decreases in primary biosynthesis (growth) generally resulted in a large decrease in secondary biosynthesis . The initial growth of toxigenic Fusaruim spp. must be prevented before harvest since nematicide residues at harvest must be within tolerances and may not be availble for inhibition of zearalenone during storage. Our present data agree with the investigation of Berisford and Ayres (1976) who found that production of zearalenone was completely inhibited in culture by the insecticide naled. On the other hand, Draughon and Churchville (1985) showed that when field corn was inoculated after silking with F. roseum and then treated with fonofos, carbaryl or maneb a significant reduction in zearalenone production was obtained.These observations are in agreement with present data (Table 3) where on application of nematicides in soils decreased the zearalenone concentrations in soils, roots and tomato fruits as compared with non-treat soils.

Table 1. Production of zearalenone by Fusarium species isolated from the untreated soils roots and fruits of tomato plants

Fusarium species@	Soils				Roots				Fruits			
	% occur*.	No. isolates tested	No. positive isolates	Conc. zear. (ppm)	% occur*.	No. isolates tested	No. positive isolates	Conc. zear. (ppm)	% occur*.	No. isolated tested	No. positive isolates	Conc. zear. (ppm)
F. avenaceum	60	4	0	0	70	4	0	0	0	0	0	0
F. culmorum	45	2	2	670	40	2	2	100	0	0	0	0
F. graminearum	70	5	5	800	80	3	3	500	80	3###	3	570
F. moniliforme	70	6	0	0	70	1	0	0	20	3##	0	0
F. nivale	30	3	0	0	35	2	0	0	10	2##	0	0
F. oxysporum	80	4	4	400	65	3	3	300	70	5###	5	265
F. solani	40	5	0	0	60	6	0	0	60	4##	0	0
Total		29	10			21	8			17	8	

@ According to Booth's nomenclture (Booth, 1971).
* number of samples tested were 15. % = number of samples containing listed specias/15 x 100
Isolated from highly decayed tomato fruits.
Isolated from moderately decayed tomato fruits.

Table 2. Effect of different nematicides in soil on zearalenone production by Fusaruim species isolated from roots, and fruits of tomato .

	No. of positive isolates / No. of isolates (Range of zearalenone / ppb)											
	Fenamiphos				Carbofuran				Aldicarb			
Fusarium species	Roots		Fruits		Roots		Fruits		Roots		Fruits	
F. avenaceum	0/4	(0)	0/0		0/0		0/0		0/0		0/0	
F. culmorum	0/2	(0)	0/0		0/0		0/0		0/0		0/0	
F. graminearum	1/3	(100)	1/3###	(450)	0/0		1/2##	(280)	0/0		0/2##	(0)
F. moniliforme	0/0	(0)	0/2##	(0)	0/1	(0)	0/2###	(0)	0/0		0/2###	(0)
F. nivale	0/0	(0)	0/0		0/2	(0)	0/0		0/2	(0)	0/0	
F. oxysporum	0/3	(0)	1/4##	(350)	1/3	(300)	1/4##	(300)	0/3	(0)	1/4###	(180)
F. solani	0/6	(0)	0/5##	(0)	1/3	(200)	0/5###	(0)	0/3	(0)	0/5##	(0)
Total	1/18 6 %		2/14 21%		2/9 22%		2/13 15%		0/8 0%		1/13 8%	

Isolated from highly decayed tomato fruits.
Isolated from moderate decayed tomato fruits.

Under the conditions of this study, the organophosphate nematicide fenamiphos and the carbamate nematicides carbofuran and aldicarb reduced the occurrence of toxigenic Fusarium spp. naturally contaminating roots and fruits of tomato plants. Zearalenone production was inhibited or reduced at harves as compared with the amount of zearalenone produced in controls. Future research should address the economic feasibility of using specific nematicides and other compounds for reducing the zeralenone contamination of human foods and animal feeds.

Table 3. Effect of nematicides on Zearalenone production naturally contaminating soils, roots and fruits of tomato plants.

No. of positive samples / No. of tested samples (Range of zearalenone/ppm)											
Control			Fenamiphos			Carbofuran			Aldicarb		
Soil	Roots	Fruits	Soil	Roots	Fruits	Soil	Roots	Fruits	Soil	Roots	Fruits
8/15 (350)	6/15 (175)	4/15 (80)	5/15 (90)	2/15 (45)	2/15 (25)	4/15 (35)	2/15 (15)	1/15 (10)	2/15 (25)	0/15 (0)	0/15 (0)

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