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EFFECT OF SOME PESTICIDES AND ANTIBIOTICS ON *Trichoderma* SPECIES

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ABSTRACT

The effect of some pesticides (fungicides, insecticides, nematicides and herbicides) as well as some antibiotics and plant oils was studied on the growth of *Trichoderma harzianum* and *T. legnorum* under both laboratory and greenhouse conditions.

The fungicides Benlate and Vitavax were the most effective ones against the two species of *Trichoderma*, they were followed by the herbicide Afalon, while the inhibition effect of the herbicide Cotoran was lower than those of the previous pesticides. The inhibition effects of the insecticides and nematicides were less than those of the used fungicides under both laboratory and greenhouse conditions.

Antibiotic Canestien showed a significant inhibition on the growth of the bioagent *Trichoderma*. But Chlorotetracycline did not inhibit the studied bioagents. The garlic oil showed moderate inhibition of the growth of the bioagent.

INTRODUCTION

Several mycoparasites belonging to the filamentous fungal genus *Trichoderma* are promising for the biological control of plant pathogenic fungi. When planning the application of antagonistic *Trichoderma* strains for the biological control, it is very important to consider the environmental parameters affecting the biocontrol agent in the soil.

Within the frames of a complex integrated plant protection, we may have to combine *Trichoderma* strains with chemical pesticides, Abd El-Moity *et al.* (1982) reported that after prolonged and repeated exposure of *T. harzianum* isolates T.14, Wt-6 and Th.1 (Egyptian isolate) to benomyl 0.5-10 Mg a.i. 1 ml no tolerance of these isolates was observed. They pointed out that continuous exposure of wide strain of *T. harzianum* to fungicides resulted in some morphological changes in colony characters, e.g. pattern of sporulation, sporadical sporulation on the agar, and changes in spore colour. Abd-Malek *et al.* (1994) found that the two herbicides Alachlor and Haloxyfop each at 100, 500 and 1000 mg/L reduced the radial growth of some fungi including *T. harzianum*. Kay and Stewart (1994) evaluated the sensitivity of four antagonists (*Chaetomium globosum*, *Trichoderma harzianum*, *T. viride* and *Trichoderma* sp.) to six fungicides, the fungi were sensitive to benomyl and the two dicarobximides. Figueras-Roca *et al.* (1996) studied the sensitivity of the nine isolates belong to five *Trichoderma* species. The experimental results showed highly significant differences among both isolates and fungicides, the fungicides: Flutrialfol, Fenarimol, and Myclobutanil were the least effective in inhibiting growth of *Trichoderma* isolates, while Kay and Stewart (1994) found that the effect of most pesticides differ from pesticides to another from slow, moderate and high reduction against antagonistic fungi, but some antagonistic fungi can tolerate the two pesticides.

McLean *et al.* (2001) found that *Trichoderma harzianum* was sensitive to Mancozeb and Thiram and less sensitive to Procymidone and Captan, while Figaeras-Roca *et al.* (2004) recorded that the toxicity of fungicides to the radial growth of *Trichoderma* differed greatly according to the type of fungicides. But Sirnivasulu *et al.* (2002) declared that all tested fungicides except zinc sulphate inhibited the growth of all *Trichoderma* spp., suggesting the non-combined use of fungicides and biological control agent.

Sesan *et al.* (1998) studied the *in vitro* action of 20 pesticides (4 fungicidal mixtures, one insecticidal mixture, 4 insecticidal-fungicidal mixtures, and 11 herbicides alone or mixed) on three antagonistic fungi: *Trichoderma viride*, *Trichothecium roseum*, and *Glicoladium roseum*. Among these compounds, five pesticides exhibited strong inhibitory activities on all test-fungi, while six showed inhibitory activity only against two test-fungi: *T. roseum* and *G. roseum*. Some pesticides tested (Tiracrab 60 PTS, Tiracrab 600 SC, Tiramet 60 PTS, Icedin Super RV and Gammavit 85 PSU) were moderately inhibitory against all three antagonistic fungi. Five products have moderately toxic action against all three antagonistic fungi. Five products have moderately toxic activity against only two antagonistic fungi. Only few pesticides were slightly inhibitory or non-inhibitory/selective on two antagonistic fungi.

Therefore, it is important to collect information and to study the effect of pesticides on the bioagents' strains.

MATERIALS AND METHODS

Laboratory experiments:

Various selected chemical pesticides Table (1) and some biological materials were tested through consecutive experiments *in vitro* (lab. Exp.). The used chemical pesticides were two fungicides, two herbicides, two insecticides and two nematicides prepared as solutions in water.

The biological materials, i.e. antibiotics and plant originated oils were dissolved in acetone before preparing their water solutions. The two *Trichoderma* isolates were kindly received from Institute of Plant Pathology, A.R.C., Giza, Egypt. Potato dextrose agar medium was supplemented with certain concentration of each tested material. Petri dishes containing material supplemented medium was incubated with PDA culture discs (4 mm diam.) of either antagonistic fungus.

The medium was inoculated with either antagonistic fungus without any pesticides as control. All plates were incubated on 20-25°C for 7 days. The linear growth of all treatments were measured after the incubation and the means of reduction percentages were calculated. Two isolates of *F. solani* and *R. solani* were previously isolated from diseased roots of cowpea plants showing severe root rotting.

Pathogenicity tests of these isolates proved their highly virulence against cowpea plants.

Table (1): Commercial and common names and chemical composition of the used fungicides, insecticides and herbicides.

Commercial Name	Common name	Chemical composition
Vitavax	Carboxin	5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide.
Benlate	Benomyl	Methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate.
Afalon	Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea.
Cotoran	Fluometuron	1,1-dimethyl-3-(a,a,a-trifluoro-m-tolyl) urea.
Vydate	Oxamyl	Methyl N'N'-dimethyl-N-[(methylcaramoyl)oxy]-1-thiooxamimidate.
Nemacur	Fenamiphos	Ethyl 3 methyl-4-(methylthio) phenyl (1-methylethyl) phosphoramidate.
Hostathion	Triazophos	O,O-diethyl o-1 phenyl-1H-1,2,4-triazol-3-yl phosphorothioate.
Selecron	Profenos	o-(4-bromo-2-chlorophenyl) o-ethyl S-propyl phosphorothioate.

Greenhouse experiments:

Autoclaved soil was placed in black plastic pots (25 cm diam.) and infested with mixture of inoculum of highly virulent isolates of *Rhizoctonia solani*, and *Fusarium solani* prepared by incubation for 14 days at 25°C on sterilized millet grains at infestation rate of 0.5 g per 100g of soil, 7 days before planting, the two *Trichoderma* isolates were grown on autoclaved wheat bran of soil, for two weeks and added to the soil at a rate of 0.5g per 100 g two days before planting. Pesticides were applied as formulated products at the recommended rates.

The nematicides (in the form of granules) were spread over the soil surface, just before planting and thoroughly mixed with the surface soil according to Chatto and Padhyay (1980). But the herbicides and insecticides were prepared as water solutions and sprayed on the soil surface before planting (El-Sheshtawi *et al.*, 1990).

The fungicides were used as seed-coating. Seeds of cowpea were planted at a rate of 15 seed per pot. The check pots (4 replicates) were used without any chemicals or biological agents. Percentages of emergence and standing plants were recorded after 15 and 45 days from planting, respectively.

RESULTS AND DISCUSSION

A. Laboratory experiments:

Data in Table (2) indicated that the most effective pesticides against the two studied bioagents were the fungicides Vitavax and Benlate, followed by the herbicide Afalon. The insecticide Selecron was more effective than Hostathion however, it was lower than the fungicidal inhibition effect. The effect of the two nematicides (Vydate and Nemacur) was somewhat similar to the effect of insecticides where the reduction percentages of the radial growth of the bioagent ranged between 50-60% with Vydate and 55-60% with

Nemacur. The results were in agreement with those of Kay and Stewart (1994), where they found that the effect of most pesticides differed from one pesticide to another from high, moderate and reduction against the antagonistic fungi. Similar results were obtained by Sesan *et al.* (1998) who studied the effect of 20 pesticides (4 fungicides, 4 insecticides and 11 herbicides) against three antagonistic fungi (*Trichoderma viride, Trichoderma roseum* and *Giliocladium roseum*).

Table (2): Inhibition effect of different pesticides against *T. harzianum* and *T. legnorum in vitro.*

Materials	Conc. in ppm	*Trichoderma legnorum*		*Trichoderma harzianum*	
		R.G.	R.P.%	R.G.	R.P.%
Control	**0**	90		90	
Vitavax	**2000**	11	87.8	10	88.9
	3000	10	88.9	9	90.0
	4000	9	90.0	7	92.2
Benlate	**500**	4	95.6	4	95.6
	1000	4	95.6	4	95.6
	1500	4	95.6	4	95.6
Afalon	**1500**	15	83.3	15	83.3
	2500	13	85.6	15	83.3
	3500	12	86.7	13	85.6
Cotoran	**1500**	65	27.8	70	22.2
	2500	60	33.33	66	26.7
	3500	55	38.9	60	33.3
Vydate	**3000**	60	33.3	60	33.3
	4000	55	38.9	60	33.3
	5000	50	44.4	55	28.9
Nemacur	**3000**	65	27.8	60	33.3
	4000	66	33.3	60	33.3
	5000	55	38.9	58	35.6
Hostathion	**3000**	70	22.2	75	16.7
	4000	65	27.8	70	22.2
	5000	60	33.3	60	33.3
Selecron	**3000**	60	33.3	70	22.2
	4000	55	38.9	66	26.7
	5000	50	44.4	60	33.3
L.S.D.		3.6		4.7	

R.G. = Radial growth (in mm.).
R.P. = Reduction percentage.

2. Effect of biological materials:

Data presented in Table (3) show that few materials had a marked antifungal effect, *in vitro*.

Data concerning the two antibiotic materials and plant originated oils, i.e. Chlorotetracycline, Canestien, mustard oil and garlic oil (Table 3) show the following:

a. Cholorotetracycline had no antifungal effect against the two *Trichoderma* spp., while Canestien showed great inhibition of the two biological agents.
b. Plant originated oils: Weak inhibition effect of mustard oil while garlic oil gave moderate effect against the two *Trichoderma* spp., where inhibition percentages ranged between 44.9% to 55.6% reduction in the case of garlic oil on both *Trichoderma* spp. These results were in agreement with Ibrahim (1999), who found that increase of garlic oil concentrations resulted in obvious inhibition of growth of most *Trichoderma* spp.

Table (3): Antifungal effect of some antibiotics and plant oils against two *Trichoderma* spp. *in vitro*.

Materials	Conc. In ppm	*Trichoderma legnorum*		*Trichoderma harzianum*	
		R.G.	R.P.%	R.G.	R.P.%
Control	**0**	90	-	90	-
Chlorotetra-cycline	**1000**	90	0.00	90	0.00
	2000	90	0.00	90	0.00
	3000	90	0.00	90	0.00
Canestien	**100**	15	72.2	22	75.6
	200	22	75.6	20	77.8
	300	20	77.8	20	77.8
Mustard oil	**250**	80	11.1	78	13.3
	500	75	16.7	75	16.7
	1000	73	18.9	70	22.2
Garlic oil	**300**	50	44.4	45	50.0
	400	45	50.0	40	55.6
	500	40	55.6	40	55.6

R.G. = Radial growth (in mm.).
R.P. = Reduction percentage.

In the greenhouse experiments using autoclaved soil infested with mixture of two highly virulent isolates of *R. solani* and *F. solani*, both of the two *Trichoderma* spp. significantly decreased disease incidence and increased the percentages of emergence and healthy plants of cowpea after 30 days of planting. On the other hand the pesticides and biological materials decreased the effect of *Trichoderma* spp. on the percentages of emergence and healthy plants except for the fungicides, herbicides (Afalon), antibiotic (Canestien) and garlic oil which increased the effect of *Trichoderma* spp., where they gave the best results 90, 100, 90, 80 and 80 emergence percentages, respectively and 100, 100, 100, 87.5 and 87.5 healthy plants, respectively when they were used with *T. harzianum*, Table (4). While other pesticides and biological material gave moderate effect on the emergence percentages and healthy plants. On the other hand, the results of *T. legnorum* were near to the results of *T. harzianum* with somewhat lower effect. These results are in agreement with those of Moustafa Mahmoud *et al.* (1996) where they reported that the efficacy of biological agent could be reduced by the type of the pesticides used in chemical management of other pests.

Table (4): Effect of certain pesticides and biological materials on the efficiency of *Trichoderma harzianum* on diseases caused by a mixture of *R. solani* and *F. solani* on cowpea plants under greenhouse conditions.

Materials	Rate of usage	Seed germination 15 days after planting		% Seedling diseased	% Healthy seedlings
		No. of emerged seedlings	% Germination		
Control 1		9	90.0	86.6	33.3
Control 2		8	80.0	0.0	100.0
Vitavax	3g/kg	9	90.0	0.0	100.0
Benlate	1g/kg	10	100.0	0.0	100.0
Afalon	2.5g/l	9	90.0	11.1	88.9
Cotoran	3g/l	7	70.0	14.2	85.8
Vydate	4ml/l	8	80.0	12.5	87.5
Nemacur	4g/l	6	60.0	16.6	83.4
Hostathion	4ml/l	7	70.0	14.2	85.8
Selecron	3ml/l	7	70.0	14.2	85.8
Chlorotetracycline	2g/l	6	60.0	16.6	83.4
Canestien	0.2ml/l	8	80.0	12.5	87.5
Mustard oil	0.5ml/l	7	70.0	14.2	85.8
Garlic oil	0.5ml/l	8	80.0	12.5	87.5

Cont. 1= pots were artificially inoculated with the pathogenic fungi and treated with the pesticides without the biological agent.
Cont. 2= pots were artificially inoculated with the pathogenic fungi and the biological agent without treating with the pesticides or biological sources.

Table (5): Effect of certain pesticides and biological materials on the efficiency of *Trichoderma legnorum* on diseases caused by a mixture of *R. solani* and *F. solani* on cowpea plants under greenhouse conditions.

Materials	Rate of usage	Seed germination 15 days after planting		% Diseased seedlings after 30 days	% Healthy seedlings after 30 days
		No. of emerged seedlings	% Germinat-ion		
Control 1		3	30	66.6	33.3
Control 2		8	80	0.0	100
Vitavax	3g/kg	9	90	0.0	100
Benlate	1g/kg	10	100	0.0	100
Afalon	2.5g/l	8	80	12.5	87.5
Cotoran	3g/l	5	60	13.6	83.4
Vydate L	4ml/l	6	70	14.3	85.7
Nemacur	4g/l	4	60	16.7	83.3
Hostathion	4ml/l	6	70	14.3	85.7
Selecron	3ml/l	5	70	14.3	85.7
Chlorotetracycline	2g/l	5	70	28.6	71.4
Canestien	0.2ml/l	6	60	0.0	100
Mustard oil	0.5ml/l	6	70	14.3	85
Garlic oil	0.5ml/l	7	70	16.7	83.3

Cont. 1= pots were artificially inoculated with the pathogenic fungi and treated with the pesticides without the biological agent.
Cont. 2= pots were artificially inoculated with the pathogenic fungi and the biological agent without treating with the pesticides or biological sources.

The also added that the herbicide oxyfluorfen significantly reduced the efficacy of biological agent *Trichoderma* spp. on cotton where it decreased percentages of emergence and survived plants. However, these particular herbicides showed no significant effect in the check treatments (without biological agent).

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تأثير بعض المبيدات والمصادر الحيوية على بعض الفطريات المضادة (تريكودرما هارزيانم وتريكودرما ليجنورم)

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تم دراسة تأثير بعض مبيدات الآفات (مبيدات فطرية ، مبيدات حشرية ، مبيدات نيماتودية ومبيدات حشائش) وبعض المواد الحيوية مثل المضادات الحيوية والزيوت النباتية على النمو الميسليومى لنوعين من *Trichoderma* وذلك فى المعمل. كما تم دراسة تأثير تلك المواد على الفطرين تحت ظروف الصوبة من حيث تأثيرها على نسبة الإنبات ونسبة النباتات السليمة بعد العدوى بخليط من فطرين ممرضين *F. solani & R. solani* .

وكانت النتائج كالآتى:

أولاً: فى المعمل:

أظهر تأثير المبيدات الفطرية (فيتافاكس وبنليت) المثبط لنمو الفطريات المضادة حيث أعاقت النمو لدرجة عالية فى المبيد الفطرى بنليت ونمو ضعيف فى حالة المبيد الفطرى فيتافاكس حيث أعطى من ٩٢,٢-٨٧,٨٤% كنسبة تثبيط أما فى حالة مبيدين الحشائش أفالون وكوتوران كان تأثير المبيد الأول أقل تثبيط مقارنة بالمبيدات الفطرية من ٨٣,٣-٨٦,٧% تثبيط وكان تأثير المبيد كوتوران أعطى نسبة تثبيط تتراوح ما بين ٢٢,٢-٣٨,٩% لكلا الفطرين ، أما فى حالة مبيدات الحشائش كان التأثير ضعيفا أيضا وكذلك بالنسبة للمبيدات النيماتودية ولكن كان تأثير السيليكرون أقوى من الهوستاثيون (حشرى) والفايديت ل أقوى من النيماكيور (نيماتودى).

وعموما كان تأثير كل المواد المستخدمة أقوى على الفطر *Trichoderma harzianum* عن تأثير تلك المواد على الفطر المضاد *T. legnorum* . أما فى حالة المصادر الحيوية إنعدم ظهور أى تأثير للمضاد الحيوى كلوروتتراسيكلين ولكن المضاد الحيوى كانستين أعطى نسبة تثبيط عالية تتراوح فى كلا الفطرين من ٧٢,٢-٧٧,٨% يلى ذلك زيت الثوم حيث أعطى نسبة تثبيط متوسطة تتراوح ما بين ٤٤,٤-٥٥,٦% أما زيت الخردل فقد أعطى نسبة تثبيط للفطرين المضادين ضعيفة تتراوح ما بين ١١,١-٢٢,٢%.

ثانيا: تحت ظروف الصوبة:

فى حالة دراسة تأثير تلك المبيدات على الفطريات المضادة وذلك من حساب وقياس نسبة الإنبات ونسبة النباتات السليمة فقد وجد أن المبيدين الفطرين وكذلك المبيد الحشائش أفالون والمضاد الحيوى كانستين وزيت الثوم أعطى أعلى نسبة تأثير.