J. Biol. Control, 10:111-115, 1996

Effect of certain soil-insecticides on *in vitro* growth, sporulation and cellulase production by *Trichoderma harzianum* Rifai.

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ABSTRACT : Three commonly used soil insecticides *viz*., BHC, phorate and carbofuran meant for the control of soil borne pests were studied for their *in vitro* influence on the growth, sporulation and cellulase production by *Trichoderma harzianum*. The insecticides were tested at 5, 10, 50, 100 and 500 ppm concentrations. Among the insecticides, phorate and carbofuran caused maximum reduction of mycelial biomass. Sporulation was strongly inhibited by BHC when compared to other insecticides. The production of cellulases was drastically reduced by incorporation of phorate.

KEY WORDS : Cellulase production, *in vitro* growth, soil-insecticides, sporulation, *Trichoderma harzianum*

Trichoderma harzianum Rifai. has been widely used for the successful management of soil borne pathogens of crops (Mukhopadhyay, 1987). The growth of *Trichoderma* in soil is influenced by various factors, and prime among them is the use of synthetic pesticides and chemicals applied to soil for plant protection (Baker, 1981). The insecticides applied to soil may have direct influence on the growth and activity of *Trichoderma* which is either native or introduced. The present study was conducted to investigate the impact of certain commonly used soil insecticides on the *in vitro* growth and activity of *Trichoderma harzianum*.

MATERIALS AND METHODS

The studies were conducted at the Department of Plant Pathology laboratory, Annamalai University, during June, 1996.

Sterilized Czapek's broth (50 ml) incorporated with varying concentrations (0, 5, 10, 50, 100 and 500 ppm) of the pesticides *viz.*, BHC (γ-isomer), carbofuran and phorate individually were inoculated with 8 mm mycelial disc of the plate culture of *T. harzianum* and incubated for 10 days at 28°C. The fungal biomass was separated through filtration and dry weight recorded at the end of the incubation period.

Sterilized Czapek's agar medium incorporated with pesticides as mentioned earlier was prepared and poured into sterile petri dishes at 20 ml volumes. They were inoculated with 8 mm fungal discs of *T. harzianum* and radial growth measured periodically up to 96 h.

Twenty five ml of sterilized Czapek's broth incorporated with varying concentrations of insecticides were prepared and inoculated as mentioned earlier. The flasks were incubated for 10 days at 28°C. The contents of the flasks were shaken and spores harvested. The spores were counted in a haemocytometer after appropriate dilution.

Czapek's broth devoid of sucrose, supplemented with cellulose powder (1%), incorporated with varying concentrations of insecticides, were inoculated for the production of cellulase. After incubation for 10 days the culture filtrate was separated, centrifuged (3000g for 20 min at 2°C) and used for enzyme assay. The activity of cellulases was measured using filter paper as the substrate (Sadasivam and Manickam, 1992).

RESULTS AND DISCUSSION

The *in vitro* mycelial biomass growth was significantly affected by the incorporation of insecticides (Table 1). The biomass yield decreased with increase of

Table 1. Effect of different insecticides on the *in vitro* growth of *T. harzianum*

Insecticide	Mycelial dry wt. (mg) at different concentrations in ppm				
	5	10	50	100	500
BHC	320 b	290 b	180 c	120 c	90 bc
Phorate	270 d	250 c	180 c	110 c	75 c
Carbofuran	300 c	280 b	200 b	150 b	100 b
Control	520 a	520 a	520 a	520 a	520 a

	SEM ±	CD (P=0.05)
Insecticides	2.97	8.44
Concentrations	3.47	9.88
Insecticides x Concentrations	6.88	19.58

concentration of chemicals. The minimum biomass production was noticed in phorate (75 mg) followed by BHC (90 mg) and carbofuran (100 mg) at 500 ppm. However, in the linear growth assay (Table 2) feeble growth (19 mm) was seen at 500 ppm in the case of BHC, whereas growth was almost nil in phorate and carbofuran at 500 ppm.

seen even up to 50 ppm concentration of all the chemicals. Addition of BHC strongly inhibited the sporulation of antagonists which was followed by carbofuran and phorate.

The production of cellulases was drastically affected by the insecticides tested. The effect was moderate up to 50

Table 2. Effect of different insecticides on the linear growth of *T. harzianum*

Insecticide	Linear growth (mm) at different concentrations in ppm				
	5	10	50	100	500
BHC	90 a	90 a	71 b	64 b	19 b
Phorate	70 c	50 b	33 d	15 d	9 c
Carbofuran	75 b	50 b	35 c	19 c	9 c
Control	90 a	90 a	90 a	90 a	90 a

	SEM ±	CD (P=0.05)
Insecticides	0.01	0.02
Concentrations	0.26	0.73
Insecticides x Concentrations	0.46	1.31

The sporulation of *T. harzianum* was strikingly affected due to incorporation of insecticides (Table 3). Sporulation was

ppm (Fig.1). At higher concentrations the cellulase production ceased and at 500 ppm it was almost nil. Maximum inhibition was

Table 3. Effect of different insecticides on the sporulation of *T. harzianum*

Insecticide	Mean spore count (x 10^6/ml) at different concentrations in ppm				
	5	10	50	100	500
BHC	4.6 d	3.9 b	2.3 c	0.9 d	0.08 d
Phorate	5.7 b	3.2 c	2.8 b	2.3 b	0.42 b
Carbofuran	5.0 c	3.0 d	2.1 d	1.2 c	0.25 c
Control	10.2 a	10.2 a	10.2 a	10.2 a	10.20 a

	SEM ±	CD (P=0.05)
Insecticides	0.01	0.03
Concentrations	0.03	0.07
Insecticides x Concentrations	0.05	0.13

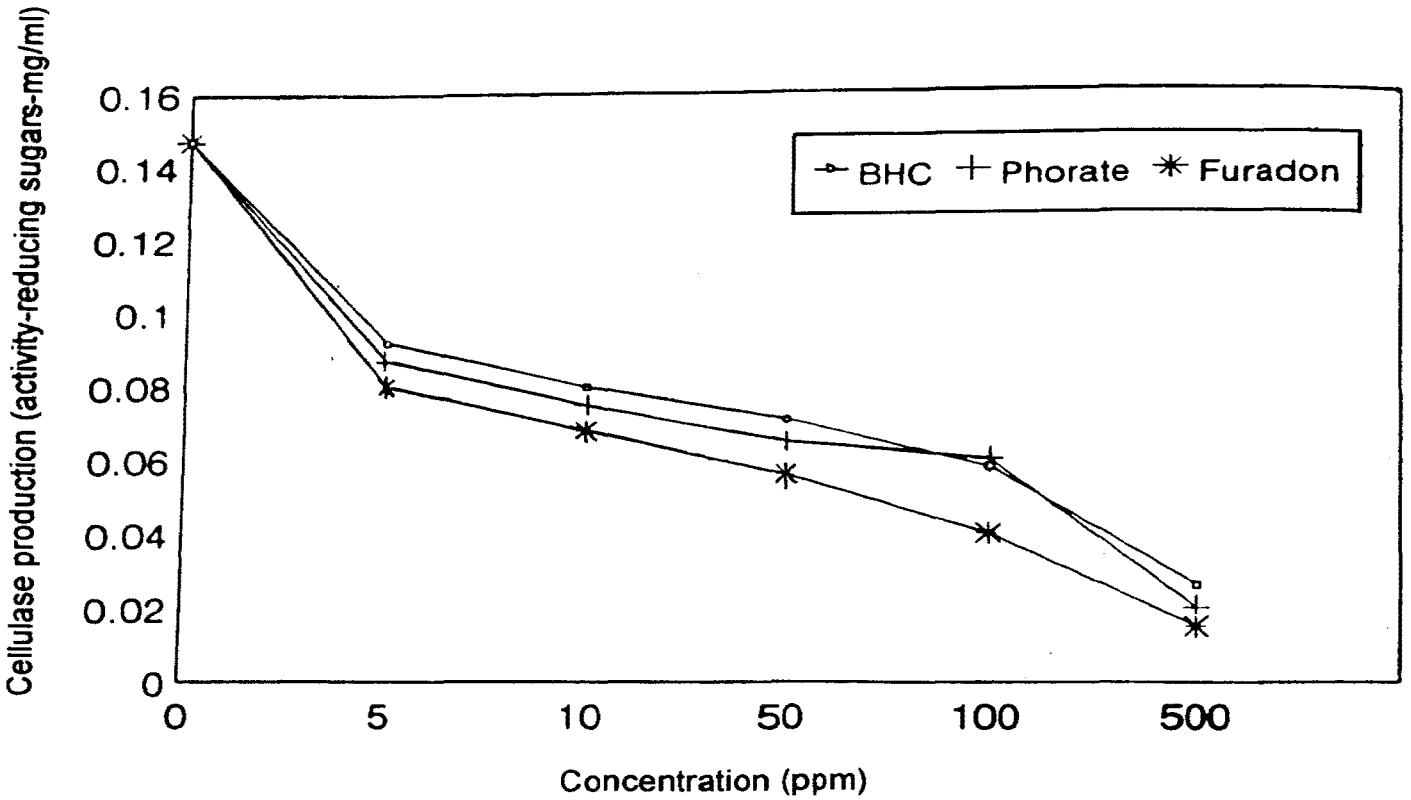

Fig.1. Effect of certain insecticides on the production of cellulase by *T. harzianum*

seen in the case of carbofuran followed by phorate and BHC.

The inhibitory effects of insecticides to *Trichoderma* in general has not been studied widely. Domsch *et al.* (1980) reported the poor growth and activity of *T. harzianum* in the presence of insecticides like aldicarb,ethoprop and sebufos. However, precise reports are not available with regard to toxic effect of BHC, carbofuran and phorate. The growth of *Trichoderma* as well as other soil fungi were strongly affected by methyl pyrimifos and chlorpyriphos (Martinez Toledo *et al.*, 1992). A similar effect has been observed in the present studies with carbofuran and phorate.

The results confirm the strong toxic effect of all the three insecticides tested emphasizing the restricted application of these insecticides to sustain growth and biocontrol potential of *Trichoderma* in the applied areas. However, the mechanism of action of individual chemicals on the physiology of *Trichoderma* has to be studied in detail.

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