Journal of Spices and Aromatic Crops

Compatibility of phorate and chlorpyriphos with *Trichoderma harzianum* for integrated disease management in black pepper

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Abstract

In the integrated management of diseases and pests in black pepper (*Piper nigrum* L.), chemicals such as phorate and chlorpyriphos are recommended besides biocontrol agent, *Trichoderma harzianum*. In order to understand the compatibility of *T. harzianum* with phorate and chlorpyriphos, experiments were conducted at different concentrations for each chemical considering the recommended dose (for phorate, 6-36 ppm a.i and for chlorpyriphos, 10-40 ppm). In *in vitro* studies with phorate, there was no significant difference in growth and spore production. In soil also, there was no reduction in viable colonies. Whereas with chlorpyriphos there was reduction in growth and spore production in *in vitro* studies, but in soil, there was no such inhibition, on the contrary there was increase in viable colonies. This indicates that phorate and chlorpyriphos can be safely applied with *T. harzianum*.

Key words: compatibility, phorate, chlorpyriphos, *Trichoderma harzianum*.

Introduction

Black pepper (*Piper nigrum* L.) is one of the major export oriented spice crops of India. Productivity of this crop is the lowest in India when compared to other countries. One of the reasons is the crop loss caused due to pests and diseases. Among the 17 diseases recorded, *Phytophthora* foot rot caused by *P. capsici* and slow decline caused by *P.capsici*, *Radopholus similis* and *Meloidigyne incognita* are serious (Sarma *et al.*, 1994; Anandaraj *et al.*, 1996). In addition to this there are reports of occurrence of mealy bugs on young vines (Anonymous, 1998). *Phytophthora* foot rot, slow decline and mealy bugs are soil-borne and not amenable to a single method of control. An integrated disease management programme involving phytosanitation, cultural, chemical and biological control measures are followed (Sarma and Anandaraj, 1998). Since slow decline is caused both by plant parasitic nematodes and *P.capsici*, phorate is used to control nematodes whereas antagonistic *Trichoderma* spp. is applied to soil to prevent population build up of *P.capsici* (Sarma *et al.*, 1996, Santhosh and Ramana, 1996). In recent years, mealy bugs are recorded on black pepper roots, which require an insecticidal application. Preliminary results indicate the efficacy of chlorpyriphos and quinolphos (Anonymous, 1998). In integrated disease management, chemicals are applied along with biocontrol agents. One advantage of combining plant protection chemicals with the biocontrol agents in the integrated biological/chemical treatment is that the pathogen can be controlled under climatic condition beyond the effective range of bioprotectant. Even reduced amount of pesticide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist. Besides diverse group of pathogens require specific chemicals for effective management. Therefore, the biocontrol agents need to be a fungicide-resistant/tolerant for use in integrated disease management. It is possible to develop such tolerant/resistance isolate by mutation or selection on pesticide containing

media. Moreover, captan, chlorothalonil, chloroneb and PCNB were not inhibitory to *Trichoderma* (Abd El moity *et al.*, 1982). Papavizas (1981) demonstrated the compatibility nature of metalaxyl with *T.harzianum* in pea seeds. Potassium phosphonate has been proved to have no adverse effect on beneficial soil microbes and it was compatible with *T. harzianum* (Wongwathanarat and Sivasithamparam, 1991). In black pepper its compatibility was proved *in vitro* by Rajan and Sarma, 1997). This study was undertaken to understand the effect of the chemicals namely phorate and chlorpyriphos on *T. harzianum* (P26) which is recommended for suppression of soil population of *Phytophthora capsici*.

Materials and Methods

In black pepper, phorate 3 g a.i /vine is recommended for the management of nematodes (Ramana, 1991). So the final concentration at the soil comes to 6 ppm of a.i by taking into account the approximate volume of soil (500 kg) a vine occupies. Hence, for *in vitro* studies, concentrations ranging from 6-36 ppm were used. In the soil upto 2000 ppm were tested. Likewise, for the management of mealy bugs, is chlorpyriphos 20 EC @ 25 ml/ 5 l/ vine is recommended (Anonymous, 1998). The final concentration of this in soil comes to 10 ppm and the compatibility was tested from 10-40 ppm *in vitro* and upto 800 ppm in soil.

The principle involved in poison food technique is to poison the potato dextrose agar (PDA) with the chemicals and then allow the biocontrol agent (BCA) to grow on the medium. PDA was prepared in 250-ml flasks and sterilised. To this medium, different quantities of chemicals were added to get final concentration. The fungicides were thoroughly mixed by shaking. Then the medium was poured into the sterile petri dishes (18-20 ml) and allowed to solidify. In each treatment, three replications were maintained. The concentrations were expressed in ppm of the commercial product available.

Two days old *Trichoderma harzianum* (P26) grown on PDA was used for this study. A disc of 6-mm dia. of the BCA was cut with a sterile cork borer and transferred aseptically in the centre of the petridish. Suitable controls were kept where the BCA is grown under same condition on PDA without chemical. The petri plates were kept at room temperature for incubation. The growth of the fungus was measured every 24 hrs. The growth of the fungus and spore production were compared with control.

The number spores produced in the petri dishes was counted using Haemocytometer. The mycelial mat along with spores was carefully transferred to 100 ml of sterile distilled water. The suspension was mixed vigorously and then filtered through the muslin cloth. A drop of the filtrate was kept over the Haemocytometer and spores were counted.

After *in vitro* studies, to test the compatibility, the experiments were carried out with soil in a plastic cups containing

~ 150 g of soil. The pesticides to be tested along with the biocontrol agent were incubated in these cups. There were three replications for each treatment. Two grams of phorate 10G was added per 100g of soil to get a final concentration of 2000 ppm. Similarly 400 µl of chlorpyriphos 20 EC was added to 100g of soil to get a final concentration of 800 ppm. For each treatment, three replications were maintained. The observations were taken on 7, 15 and 30 days after inoculation. The number of colonies were counted by taking a soil sample of 1g (moisture free basis) from each replication. The samples were plated in a petriplate containing *Trichoderma* selective medium (Elad and Chet, 1983)

Results and discussion

Results with phorate showed that there was no significant difference in growth between the control and phorate treated plates. We could notice profuse growth in phorate treated petri plates. The mycelial growth covered the entire plate within 48 hrs and sporulated at 72 hrs. As far as spore production was concerned, even though slight reduction in the spore production was noticed but the difference was not significant. Similarly in soil also, there was no significant reduction in number of viable colonies (Table 1,3). Hence at the final concentration at the rhizosphere, phorate may not affect the growth and spore production of *T. harzianum*.

Similarly experiments with chorpyriphos revealed that at 24 hrs after inoculation, there was some difference in growth. In chlorpyriphos treated plates the growth rate was low and there was a significant reduction in the spore production (Table 2). However in soil, there was no such reduction in viable population. In some treatments there was an increase of population than the control (Table 4).

In the rhizosphere, the biocontrol agent *Trichoderma* can colonise the entire root zone and offer localised protection. This is unattainable through chemical control. It would be unrealistic to expect that *Trichoderma* biocontrol agent can completely replace chemical fungicides in disease control. In this context, it is necessary to understand the compatibility nature of biocontrol agent with the pesticides. Phorate being a systemic insecticide, it has no effect on direct contact with biocontrol agent. Whereas, chlorpyriphos being a contact insecticide has some retarding effect in *in vitro* studies. But when it is used in soil, no such inhibitory effect was noticed, on the contrary an increase in population was noticed. It might be due to the utilization of phosphorus from the chemical. This study indicates the compatibility nature of phorate and chlorpyriphos with *T. harzianum*.

ACKNOWLEDGEMENTS

The authors are grateful to Indian Council of Agricultural Research (ICAR) for the financial assistance through National

network on *Phytophthora* diseases of Horticultural Crops (PHYTONET).

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Table 1. Effect of phorate on growth and sporulation of *T. harzianum.*

Sl. No.	phorate conc. in ppm	Growth of *Trichoderma* (dia) in mm, after		Spore load (X 10^7 / ml) after 96 h
		24 h	48 h	
1.	6	61.50	90.0	19.15
2.	12	59.00	90.0	18.57
3.	24	62.50	90.0	16.65
4.	36	60.17	90.0	15.53
5.	Control	59.17	90.0	19.47
CD at 0.05:		2.456		0.6684

Table 2. Effect of chlorpyriphos on growth and sporulation of *T. harzianum*.

Sl. No.	Chlorpyriphos conc.in ppm	Growth of *Trichoderma* (dia) in mm, after			Spore load (X 10^7 / ml) after 96 h
		24 h	48 h	72 h	
1.	10	30.8	67.4	90.0	9.31
2.	20	29.5	63.2	90.0	8.50
3.	30	26.3	60.0	88.0	6.68
4.	40	26.0	57.6	88.0	5.70
5.	Control	45.0	90.0	90.0	26.17
CD 0.05%		2.2	5.7		0.5548

Table 3. Growth and sporulation of *T. harzianum* when mixed with phorate in soil.

Sl No	BCA Conc	phorate conc In ppm	No. of *Trichoderma* colonies found in soil (X10 ^{5} CFU) after		
			7 Days	15 Days	30 Days
1.	1%	100	9.7	10.3	10.0
		500	7.0	10.3	9.7
		1000	5.33	4.0	4.3
		2000	3.7	7.0	6.3
		control	9.0	10.0	10.7
2.	2%	100	26.3	25.0	25.3
		500	24.3	22.7	19.7
		1000	15.3	19.7	16.3
		2000	16.7	15.0	15.7
		control	25.7	28.0	26.3
3.	3%	100	40.3	49.7	59.3
		500	26.7	31.3	36.3
		1000	23.7	27.7	25.7
		2000	22.3	30.0	29.7
		control	40.3	51.3	62.7

CFU: colony forming units

CD at 0.05: BCA : 0.43
Treatments : 0.56
Days : 0.43

BCA vs Treatments : 0.96
BCA vs Days : 0.75
Treatments vs Days : 0.96
BCA vs Treatments vs Days : 1.67

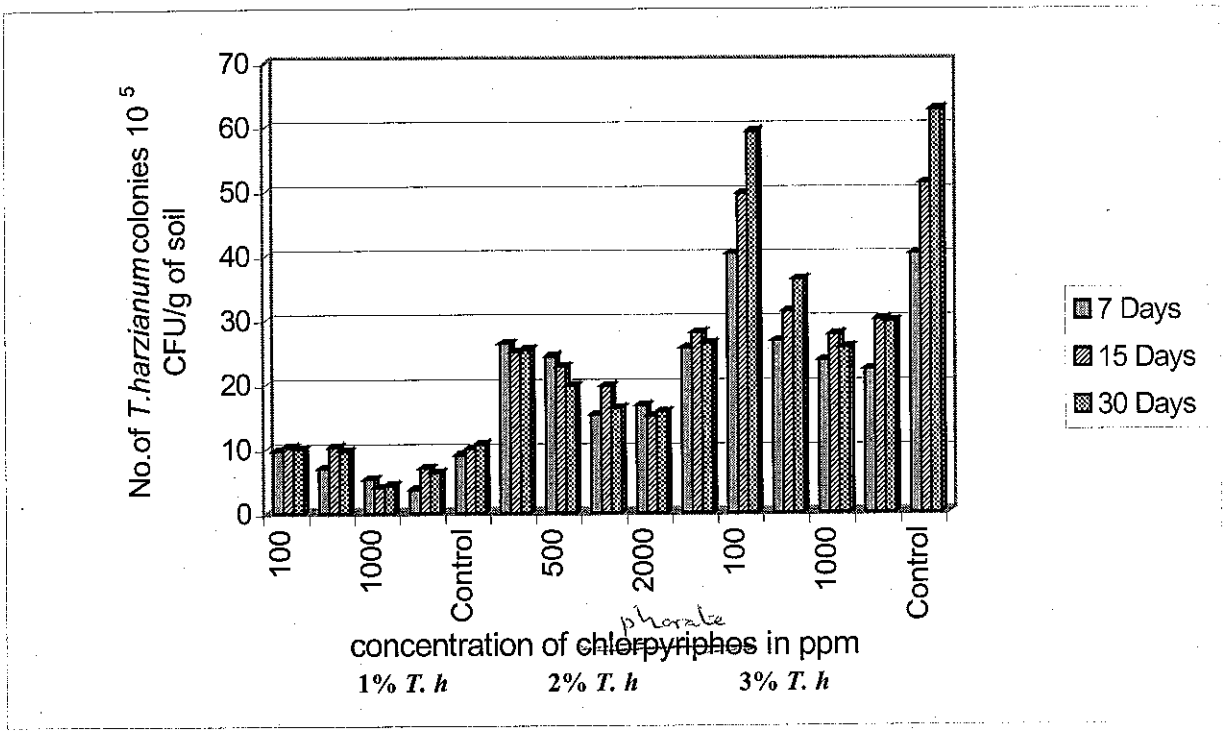
Table 4. Growth and sporulation of *T. harzianum* when mixed with chlorpyriphos in soil.

Sl No	BCA Conc	chlorpyriphos conc in ppm	No. of *Trichoderma* colonies found in soil (X10 ^{5} CFU) after		
			7 Days	15 Days	30 Days
1.	1%	200	30.3	24.7	37.3
		400	40.3	51.3	72.3
		600	36.0	37.0	38.7
		800	40.3	41.7	63.0
		control	23.2	26.1	23.1
2.	2%	200	24.3	26.0	34.3
		400	36.0	28.3	38.3
		600	30.0	25.3	27.0
		800	47.7	43.0	45.7
		control	22.7	23.7	21.7
3.	3%	200	31.0	47.3	47.7
		400	39.0	50.7	48.3
		600	36.7	45.7	52.7
		800	39.0	46.0	53.3
		control	22.3	27.3	21.0

CFU: colony forming units

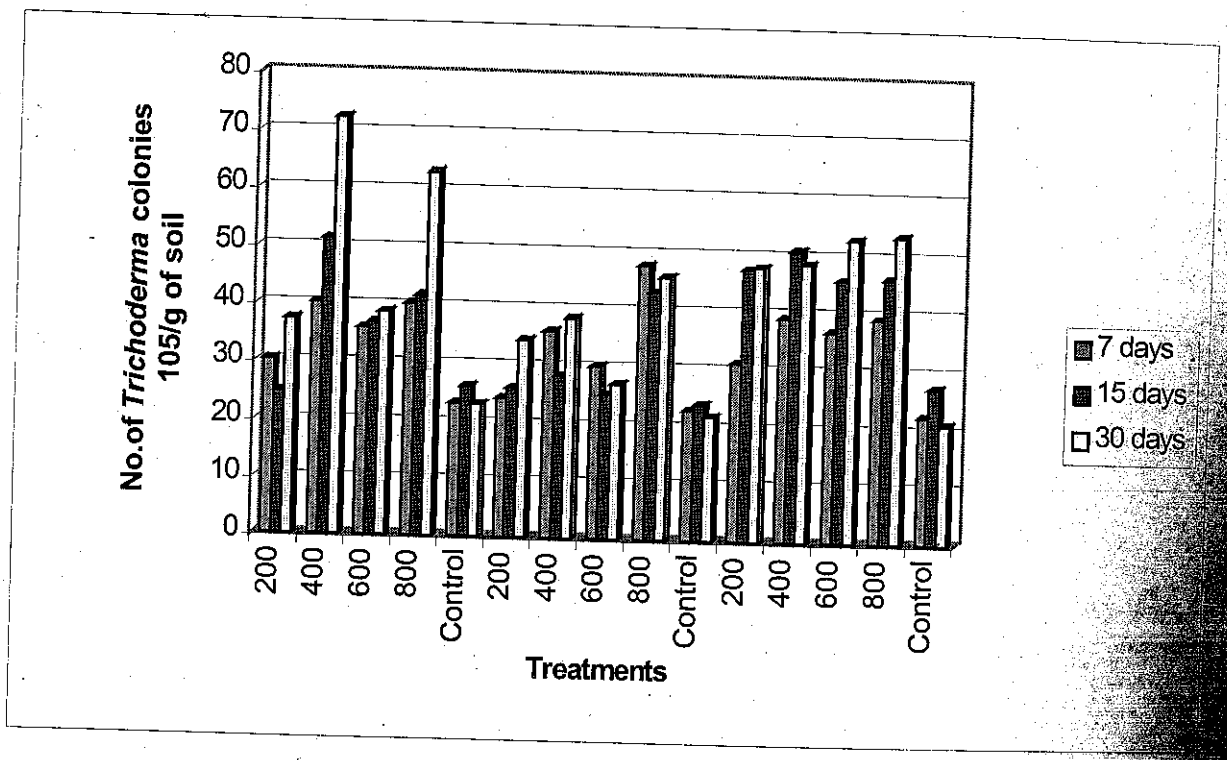
CD at 0.05:	BCA	: 0.75	BCA vs Treatments	: 1.68
	Treatments	: 0.97	BCA vs Days	: 1.30
	Days	: 0.75	Treatments vs Days	: 1.68
			BCA Vs Treatments vs Days	: 2.91

Fig. 1. Growth and sporulation of *T. harzianum* (*T. h*) when mixed with phorate in soil



CD at 0.05:	*T. h*	: 0.43	*T. h* vs Treatments	: 0.96
	Treatments	: 0.56	*T. h* vs Days	: 0.75
	Days	: 0.43	Treatments vs Days	: 0.96
			T. h vs Treatments vs Days	: 1.67

Fig. 2 Growth and sporulation of *T. harzianum* when incubated with chlorpyriphos in soil



CD at 0.05:	BCA	: 0.75	BCA Vs Treatments	: 1.68
	Treatments	: 0.97	BCA Vs Days	: 1.30
	Days	: 0.75	Treatments Vs Days	: 1.68
			BCA Vs Treatments Vs Days	: 2.91