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Compatibility of phorate and chlorpyriphos with *Trichoderma harzianum* (Rifai.) applied for integrated disease management in black pepper (*Piper nigrum* L.)[1]

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Abstract

The compatibility of the biocontrol agent, *Trichoderma harzianum* utilized for the management of *Phytophthora* foot rot of black pepper (*Piper nigrum*), with phorate and chlorpyriphos applied for the management of nematodes and mealybugs respectively, was studied. The experiments were conducted *in vitro* and in soil at different concentrations for each chemical considering the recommended dose (6–36 ppm ai for phorate and 10–40 ppm ai for chlorpyriphos). The *in vitro* studies indicated that phorate at 6–36 ppm did not affect radial growth and sporulation of *T. harzianum*, whereas, chlorpyriphos at 10-40 ppm retarded radial growth up to 50% at 24 h and 48 h but not at 72 h, and retarded sporulation. In soil, there was no significant difference in the number of viable colonies of *T. harzianum* at 1% concentration for phorate concentrations of 1000 and 2000 ppm. However, incorporation of chlorpyriphos into soil resulted in increase in number of viable colonies of *T. harzianum*. The study indicated that phorate and chlorpyriphos could be safely applied with *T. harzianum* for the management of *Phytophthora* foot rot, nematodes and mealybugs on black pepper.

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

Introduction

Among the various diseases affecting black pepper (*Piper nigrum* L.) in India, *Phytophthora* foot rot caused by *P. capsici* and slow decline caused by *P. capsici*, *Radopholus similis* and *Meloidogyne incognita* are serious (Sarma *et al.*1994; Anandaraj *et al.* 1996). In addition, root mealybug (*Planococcus* sp.) infesting black pepper is becoming serious in recent years (IISR 1998). *Phytophthora* foot rot, slow decline and root mealybug are soil-borne and not amenable to a single method of control. An integrated approach involving phytosanitation, cultural, chemical and biological control measures are adopted for the management of *Phytophthora* foot rot (Sarma & 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. are applied to soil to prevent population build up of *P. capsici* (Sarma *et al.* 1996; Eapen & Ramana 1996). Preliminary studies indicate the efficacy of drenching chlorpyriphos and quinalphos for the management of root mealybug (IISR 1998). In integrated disease management programme (IDM), chemicals are applied along with biocontrol agents. Hence biocontrol agents need to be pesticide-resistant/tolerant for use in such programmes. The compatibility of fungicides such as captan, chlorothalonil, chloroneb, PCNB, metalaxyl and potassium phosphonate to *Trichoderma* spp. has been reported (Papvizas 1981; Moiety *et al.* 1982; Wongwathanarat & Sivasithamparam 1991; Rajan & Sarma 1997). This study was undertaken to understand the effect of two pesticides namely, phorate and chlorpyriphos, on *T. harzianum* (isolate P26) which is recommended for the suppression of soil population of *P. capsici*.

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Materials and methods

In black pepper, phorate is recommended @ 3g ai/vine for the management of nematodes (Ramana 1991). Hence, the concentration of phorate in the soil is 6 ppm of ai by taking into account the approximate volume of soil (500 kg) a vine occupies. Hence, for *in vitro* studies, concentrations ranging from 6 to 36 ppm were used. In the soil, 100 to 2000 ppm concentrations were tested. Likewise, for the management of root mealybug, chlorpyriphos 20 EC is recommended @ 25 ml/ 5 1 per vine (IISR 1998) and the concentration of the pesticide in soil is 10 ppm. Hence the pesticide was tested from 10 to 40 ppm *in vitro* and 200 to 800 ppm in the soil.

For *in vitro* studies, potato dextrose agar (PDA) was prepared in 250 ml flasks and sterilized. To this medium, different quanitites of chemiclas were added to obtain the final desired concentrations. The chemicals were thoroughly mixed by shaking. The medium was then poured into sterile petri dishes (18–20 ml) and allowed to solidify. In each treatment, three replictions were maintained.

Two day old cultures of *T. harzianum* grown on PDA was used for the study. A disc of 6 mm dia. of the biocontrol agent (BCA) was cut with a sterile cork borer and transferred aseptically to the centre of the petri plate. Suitable controls were kept where the BCA was grown under the same condition on PDA but without the chemicals. The petri plates were kept at room temperature for incubation. The growth of the fungus was measured every 24 h. The growth of the fungus and spore production were compared with control. The number spores produced in the petri plates was counted using a haemocytometer.

The compatibility experiments were also carried out in soil filled (~150 g) in plastic cups. The pesticides to be tested along with the BCA were incubated in these cups. Three replications were maintained for each treatment. Two grams of phorate 10 G was added per 100 g of soil to obtain a final concentration of 2000 ppm. Similarly 400 µl of chlorpyriphos 20 EC was added to 100 g of soil to obtain a final concentration of 800 ppm. Various concentrations of the pesticides in the soil were obtained by adding suitable quantities of pesticides to the soil. Observations on number of colonies formed were taken on 7, 15 and 30 days after inoculation. The number of colonies was counted by taking a soil sample of 1 g (moisture free basis) from each replication. The samples were plated in a petri plate containing *Trichoderma* selective medium (Elad & Chet 1983).

Results and discussion

In vitro incubation of *T. harzianum* with phorate for 24 h resulted in no significant reduction in growth between control and phorate treated plates of all treatments except for 24 ppm. After 48 h, no significant reduction in radial growth was noticed. Profuse growth of mycelia was observed in phorate treated petri plates. The mycelial growth covered the entire plate within 48 h and sporulated at 72 h. However, significant reduction in spore production was noticed at 12, 24 and 36 ppm treatments but at 6 ppm treatment the difference was not significant (Table 1). In soil at 1% BCA concentration, the difference in number of viable colonies of *T. harzianum* was not significant for phorate concentrations of 1000 and 2000 ppm. In the other two BCA concentrations (2 and 3%), the differences were significant (Table 3). Since there was no significant reduction in the number of viable colonies at phorate concentrations of 100 and 500 ppm, the final concentration at the rhizosphere, the recommended dose of phorate will not affect the growth and spore production of *T. harzianum*. Among the three concentrations of BCA, 3% BCA was the best since sporulation was very high.

Similarly, *in vitro* experiments with chlorpyriphos revealed that at 24 and 48 h after inoculation, there were significant differences in radial growth of mycelia of the BCA. In untreated control, *T. harzianum* attained maximum radial growth (90 mm) by 48 h. However, in the chlorpyriphos treated plates, it took 72 h for attaining maximum

Table 1. Effect of phorate on growth and sporulation of *Trichoderma harzianum* (*in vitro*)

Phorate (ppm)	Dia. (mm) of *Trichoderma* colony after		Spore concn. (x 10^7/ml)
	24 h	48 h	after 96 h
6	61.5	90.0	19.15
12	59.0	90.0	18.57
24	62.5	90.0	16.65
36	60.2	90.0	15.53
Control	59.2	90.0	19.47
CD (P=0.05)	2.5	NS	0.67

N S = Non Significant

Table 2. Effect of chlorpyriphos on growth and sporulation of *Trichoderma harzianum* (*in vitro*)

Chlorpyriphos (ppm)	Dia. (mm) of *Trichoderma* colony after 24 h	48 h	72 h	Spore concn. (x 10^7per ml) after 96 h
10	30.8	67.4	90.0	9.31
20	29.5	63.2	90.0	8.50
30	26.3	60.0	88.0	6.68
40	26.0	57.6	88.0	5.70
Control	45.0	90.0	90.0	26.17
CD (P=0.05)	2.2	5.7	NS	0.55

N S = Non significant

radial growth. But after 96 h, the differences were not significant. In chlorpyriphos treated plates the growth rate was low and there was a significant reduction in spore production (Table 2). However in soil, there was no such reduction in viable population and on the contrary, there was a significant increase in viable colonies of *T. harzianum* in all treatments (Table 4).

Phorate being a systemic pesticide has no effect on direct contact with *T. harzianum,* whereas, chlorpyriphos being a contact insecticide has retarding effect on the growth of *T. harzianum* in *in vitro* studies. But when used in soil, no such inhibitory effect was noticed; on the contrary an increase in population of BCA was noticed which might probably be due to the utilization of

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

Conc. of *T. harzianum*	Conc. of phorate (ppm)	No. of *Trichoderma* colonies in soil (x 10^5 CFU) after 7 Days	15 Days	30 Days	Mean
1%	100	9.7	10.3	10.0	10.0
	500	7.0	10.3	9.7	9.0
	1000	5.3	4.0	4.3	4.6
	2000	3.7	7.0	6.3	5.7
	Control	9.0	10.0	10.7	9.9
2%	100	26.3	25.0	25.3	25.6
	500	24.3	22.7	19.7	22.3
	1000	15.3	19.7	16.3	17.1
	2000	16.7	15.0	15.7	15.8
	Control	25.7	28.0	26.3	27.3
3%	100	40.3	49.7	59.3	49.8
	500	26.7	31.3	36.3	31.4
	1000	23.7	27.7	25.7	25.7
	2000	22.3	30.0	29.7	27.3
	Control	40.3	51.3	62.7	52.4

CD (P=0.05) : Treatments : 0.6

T. harzianum conc. : 0.4

CFU = Colony forming units

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

Conc. of *T. harzianum*	Conc. of chlorpyriphos (ppm)	No. of *Trichoderma* colonies in soil (x 10^5 CFU) after			
		7 Days	15 Days	30 Days	Mean
1%	200	30.3	24.7	37.3	30.8
	400	40.3	51.3	72.3	54.7
	600	36.0	37.0	38.7	37.2
	800	40.3	41.7	63.0	48.3
	Control	23.2	26.1	26.7	26.2
2%	200	24.3	26.0	34.3	28.2
	400	36.0	28.3	38.3	34.2
	600	30.0	25.3	27.0	27.4
	800	47.7	43.0	45.7	45.4
	Control	22.7	23.7	21.7	22.7
3%	200	31.0	47.3	47.7	42.0
	400	39.0	50.7	48.3	46.0
	600	36.7	45.7	52.7	45.0
	800	39.0	46.0	53.3	46.1
	Control	22.3	27.3	21.0	23.6
CD (P=0.05) :	Treatments	: 1.0			
	T. harzianum conc.	: 0.8			

CFU = Colony forming units

phosphorus by the BCA from the chemical. The significant increase in *T. harzianum* population could also probably be due to reduction in the natural soil fauna such as mycophagus arthropods, which feed on fungal propagules in soil. In the absence of antagonistic fauna, the population of *T. harzianum* would have increased resulting in increased number of colonies.

Since phorate and chlorpyriphos were found to be effective for the management of nematodes and root mealybug respectively, its compatibility with *Trichoderma* spp. which is used for the management of foot rot of black pepper becomes more relevant and important for IDM in black pepper. The study indicated the compatibility of phorate and chlorpyriphos at recommended doses with *T. harzianum* thereby suggesting their potential in IDM of foot rot of black pepper.

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