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Tolerance of fungal and bacterial biocontrol agents to six pesticides commonly used in the control of soil borne plant pathogens

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The compatability of fungal (*Trichoderma harzianum*, *Trichoderma virens* and *Pochonia chlamydosporia*) and bacterial biocontrol agents (*Bacillus subtilis* and *Pseudomonas fluorescens*) was assessed to 6 pesticides viz., carbendazim, mancozeb, metalaxyl, captan, thiram, and nemacur commonly used by farmers in India for the control of soil borne plant pathogens. The compatability was assessed at different concentrations and the concentration of 60, 1050, 160, 225, 25, and 980 µg/ml of carbendazim, metalaxyl, captan, mancozeb, thiram and nemacur was the safe tolerance limit for *T. harzianum* whereas the corresponding values for *T. virens* were 40, 1000, 125, 177, 9, and 700 µg/ml, respectively. The safe tolerance limit for *P. chlamydosporia* were 37.5 µg carbendazim/ml, 75 µg captan/ml, 100 µg metalaxyl/ml, 5 µg thiram/ml, 110 µg mancozeb/ml and 250 µg nemacur/ml. Among the bacteria, *P. fluorescens* was found to be more compatible with fungicides than *B. subtilis* and the maximum tolerance concentration for the former being 2500 µg Thiram/ml, 1600 µg mancozeb/ml, and 50,000 µg/ml for captan and carbendazim. Hence, pesticidal contamination at above concentration in soil will not affect their effectiveness. Moreover, the pesticide tolerance ability broadened the use as these biopesticides in conjugation with pesticides can be applied under integrated disease management for the management of soil borne plant pathogens.

Key words: Biocontrol agents, pesticides, compatability, soil borne plant pathogens.

INTRODUCTION

Soil-borne plant pathogens are highly destructive pathogens and cause tremendous yield losses to all kinds of crops. Control of plant diseases by the use of antagonistic microorganisms can be an effective means (Cook and Baker, 1983). Interaction between biocontrol agents and plant pathogens has been studied extensively and application of biocontrol agents to protect some commercially important crops is promising (Vesseur et al., 1990). A large number of plant diseases have been successfully controlled through fungal and bacterial antagonists (Sahebani and Hadavi, 2008; Federico et al., 2007; Cook and Baker, 1983; Campbell, 1989; Vidhyasekaran et al., 1997).

Supplementation with specific compounds may provide a competitive advantage for the establishment of the introduced biocontrol agents and improve the biocontrol. In several disease management strategies, the addition of fungicide at reduced rates in combination with biocontrol agents has significantly enhanced disease control, compared to treatments with biocontrol agent

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Table 1. *In vitro* compatability of *Trichoderma harzianum, T. virens, Pochonia chlamydosporia,* with some common pesticides. MTC- Maximum tolerance concentration; MIC- Maximum inhibition concentration.

Treatment	*T. harzianum*		*T. virens*		*P. chlamysporia*	
Carbendazim	MTC*	MIC	MTC	MIC	MTC*	MIC
	60	500	40	405	37.5	250
Metalaxyl	1050	2400	1000	2200	100	500
Captan	160	1000	125	875	75	500
Mancozeb	225	755	177	625	110	350
Thiram	25	150	9	95	5	50
Nemacur	980	1150	700	1000	250	450

Each value is mean of 3 replicates; * Concentrations are in (µg/ml).

alone (Frances et al., 2002; Buck, 2004). Integrated use of biocontrol agent with reduced dose of fungicide was effective against fusarium crown and root rot of tomato (Omar et al., 2006), late leaf spot of groundnut (Kishore et al., 2005), rhizoctonia root rot, take-all disease of spring wheat (Duffy, 2000) and post harvest diseases of fruits (Chand-Goyal and Spotts, 1996) compared with the individual components of disease management. The objectives of the present study is to test the growth of different biocontrol agents with commonly used pesticides at different concentrations under *in vitro* conditions for the control of soil borne plant pathogens.

MATERIALS AND METHODS

Six pesticides viz., carbendazim (Bavistin 50 WP), mancozeb (Dithane M-45 75 WP), metalaxyl (Apron 35 SD), captan (Captaf 50 WP), thiram (TMTD 75 WP), and nemacur (Fenamiphos) were tested against the biocontrol agents using poisoned food technique (Grover and Moore, 1961). 50 ml aliquots of PDA (double strength) was taken in an Erlenmeyer flask of 250 ml capacity and sterilized in an autoclave. Different concentrations of the pesticides from 10, 25, 50, 125, 250, 500, 1000, 2000, 3000, and 5000 µg/ml were prepared in distilled water. 50 ml of a concentration was aseptically transferred to the Erlenmeyer flask containing 50 ml PDA. 5 Petri plates (90 mm diameter) for each concentration of the fungicides were prepared by pouring 20 ml PDA aliquots in each plate and allowed to solidify. Thereafter, the plates were seeded centrally with a 3 mm disc of 4 days old culture of *Trichoderma harzianum, Trichoderma virens* and *Pochonia chlamydosporia*. PDA plates without a fungicide but inoculated with the fungi served as a control. The inoculated plates were incubated at 25 ± 2°C for 5 days. The radial growth of the colony in each treatment was measured and the percent inhibition of growth was calculated by the formula:

$$I = C - T/C \times 100$$

Where, I = Percent growth inhibition; C = Radial growth in control (mm); T = Radial growth in treated plates (mm). And ED_{90} (maximum inhibition concentration) and ED_{50} (safe tolerance concentration) were determined.To determine the compatability of *B. subtilis* and *P. fluorescens* with same pesticides, 25 to 50,000 µg/ml concentrations were prepared in double distilled water. Double strength nutrient agar was used as medium for both the bacteria. 20 ml of nutrient agar containing desired concentration was poured in Petri plates and left over night to observe contamination, if any. Thereafter, 0.1 ml of overnight cultures was spread over the solidified plates with a glass spreader. The plates were incubated at 30 ± 2°C for 24 h and bacterial colonies were identified and counted.

RESULTS

The maximum growth of *T. harzianum* was inhibited at a concentration of 500, 2400, 1000, 755, 150, and 1150 µg/ml of carbendazim, metalaxyl, captan, mancozeb, thiram, and nemacur, respectively.

The corresponding value for *T. virens* were 405, 2200, 875, 625, 95 and 1000 µg/ml of of carbendazim, metalaxyl, captan, mancozeb, thiram and nemacur, respectively (Table 1).

The fungicides at concentrations of 60 µg carbendazim/ml, 1050 µg metalaxly/ml, 160 µg captan/ml, 225 µg mancozeb/ml, 25 µg Thiram/ml, and 980 µg Nemacur/ml seem to be safe tolerance limit for *T. harzianum*. For *T. virens* the safe tolerance limit were 40 µg carbendazim/ml, 125 µg captan/ml, 177 µg mancozeb/ml 1000 µg metalaxyl/ml, and 700 µg nemacur/ml (Table 1). *Pochonia chlamydosporia* showed less tolerance to the 6 pesticides tested.

The fungus was inhibited by the concentrations of 250 µg carbendazim/ml, 500 µg each of captan and metalaxyl/ml, 350 µg mancozeb/ml, 50 µg thiram/ml and 450 µg nemacur/ml. Whereas, the safe tolerance limit for *P. chlamydosporia* were 37.5 µg carbendazim/ml, 75 µg captan/ml, 100 µg metalaxyl/ml, 5 µg thiram/ml, 110 µg mancozeb/ml, and 250 µg nemacur/ml (Table 1). Biocontrol bacteria were found more tolerant to fungicides than fungi (Table 2).

The maximum tolerance concentration for *Bacillus subtilis* were 3200 µg captan/ml, 60 µg thiram/ml 600 µg mancozeb/ml. Whereas, in case of carbendazim, the bacteria showed tolerance even for a concentration of 50,000 µg/ml (Table 2).

Pseudomonas fluorescens was found to be more compatible than *B. subtilis* with fungicides, the maximum tolerance limit for the former being 2500 µg Thiram/ml, 1600 µg mancozeb/ml and 5 µg/100 ml for captan and carbendazim and 8000 µg nemacur/ml (Table 2).

Table 2. *In vitro* compatability of *Bacillus subtilis* and *Pseudomonas fluorescens* with some common pesticides.

Treatment	*B. subtilis*		*P. fluorescens*	
	MTC*	MIC	MTC	MIC
Carbendazim	50, 000	-	50, 000	-
Metalaxyl	7,000	10,000	10,000	25,000
Captan	3200	4000	50, 000	-
Mancozeb	600	1000	1600	2000
Thiram	60	100	2500	3000
Nemacur	3500	4200	8000	9000

Each value is mean of three replicates; *Concentrations are in (µg/ml); MTC, Maximum tolerance concentration; MIC, Maximum inhibition concentration.

DISCUSSION

The compatability tests revealed that the *Trichoderma* species showed more tolerance to metalaxyl as compared to other pesticides used in the study. Similar results have been obtained by other workers. Sharma et al. (2001) found that, *T. harzianum* is showing more tolerance to metalaxyl as compared to carbendazim. More or less similar results have been found by other workers also (Nallathambi et al., 2009; Mukhopadhyay et al., 1986; Mukherjee et al., 1989, Papavizas et al., 1982; Viji et al., 1997). Different workers have reported chlorothalonil, captan and captafol as tolerant for *T. harzianum* even at higher concentrations up to 2000 mg/ml in spore germination tests (Abdel-Moity et al., 1982; Papavizas et al., 1982; Mishra et al., 2004). The biocontrol bacteria viz., *Pseudomonas fluorescens* and *Bacillus subtilis* were found more tolerant to fungicides than fungi. This may be due to the reason that, some bacteria can use pesticides as nutrients and hence can tolerate higher concentrations of chemicals (Kishore and Jacob, 1987; Aislabie and Jones, 1995).

The present study clearly demonstrated that, soil borne plant pathogens can be successfully managed by combined application of biocontrol agents with a cheap fungicide like carbendazim, mancozeb, metalaxyl, captan, thiram, and nemacur commonly used by farmers in India at low doses. Also pesticidal contamination in soil will not affect the biocontrol agent effectiveness and hence can be easily applied in conjugation with the pesticides for the control of soil borne plant pathogens.

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