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Evaluation of toxicity of agrochemicals on *Trichoderma* isolates *in vitro*

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Thirty nine agrochemicals comprising of eighteen fungicides, eleven insecticides, six fertilizers and four herbicides were evaluated at different concentrations against two isolates of *Trichoderma* spp., and EC_{50} and EC_{90} values were calculated based on inhibition in radial growth. Isolate *T. virens* TV9, obtained from citrus orchard, – relatively less exposed to agrochemicals was found more sensitive compared to isolate *T. harzianum* Th4, obtained from cotton ecosystem relatively more exposed to agrochemicals. Among fungicides, benzimidazoles showed higher toxicity followed by chlorothalonil and triazoles when mean EC_{50} values were compared. Wettable sulphur, Bordeax mixture, azoxystrobin and mancozeb were found to be less toxic to *Trichoderma* spp. Among insecticides, organophosphorous group was found more toxic while carbofuran followed by spinosad were least toxic. Among fertilizers zinc sulphate and diammonium phosphate were found highly toxic where as potassium nitrate, muriate of potash and ammonium sulphate showed less toxicity. All the four tested herbicides, *i.e.*, pendimethalin, alachlor, glyphosate and 2, 4 - D were found to be toxic to *Trichoderma* spp.

Key words: Toxicity, *Trichoderma* spp., fungicides, insecticides, fertilizers, herbicides, EC_{50} and EC_{90}

INTRODUCTION

Soil borne plant pathogenic fungi such as *Fusarium, Phytophthora, Pythium, Rhizoctonia, Sclerotium* etc. cause diseases in most of the economically important crop plants. Chemical means of managing the diseases caused by these pathogens are not practicable owing to high cost of chemicals and environmental pollution. Biological control offers a novel approach when applied either alone or in combination with other management practices without the demerits of chemical control (Papavizas, 1985; Mukhopadhyay, 1987). *Trichoderma* is one of the most common soil inhabitants and extensively studied biocontrol agent in the management of soil borne plant pathogens (Elad *et al.,* 1980). Applied in the soil, *Trichoderma* proliferates in the soil and protects the crop from pathogens for a longer time.

Species of *Trichoderma* are being used either as seed treatment or soil application to manage several soil borne plant diseases. Once in the soil, the antagonist is continuously exposed to different agrochemicals applied to the field either in soil or as foliar sprays. Agrochemicals sprayed aerially reaches the soil (by means of air currents or are washed off the plant surface due to rain) and is likely to influence the efficacy of native or applied biocontrol agents like *Trichoderma*. Hence it is necessary to assess toxicity of these agrochemicals on *Trichoderma* so that suitable strategies may be formulated to protect or increase the efficacy of

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applied *Trichoderma* isolate(s) in field. The present investigation is aimed at evaluating the toxicity of agrochemicals at different concentrations on two isolates of *Trichoderma* spp.

MATERIALS AND METHODS

Two isolates of *Trichoderma* spp., *viz.*, *T. virens* TV9 (obtained from citrus orchard where in relatively lower amount of agrochemicals are used) and *T. harzianum* Th4 (obtained from cotton ecosystem where in usage of agrochemicals is more) are used in the present investigation. Eighteen fungicides, *viz.*, copper oxychloride, Bordeaux mixture, wettable sulphur, mancozeb, thiram, captan, carbendazim, benomyl, carboxin, metalaxyl, propiconazole, hexaconazole, tricyclazole, tridemorph, fosetyl-Al, azoxystrobin, chlorothalonil and dinocap, eleven insecticides *viz.*, endosulfan, chlorpyrifos, quinalphos, dimethoate, indoxacarb, carbofuran, imidacloprid, fipronil, thiamethoxam, emamectin benzoate and spinosad, seven fertilizers *viz.*, urea (46% N), muriate of potash (60% K_2O), ammonium sulphate, diammonium phosphate (DAP), potassium nitrate and zinc sulphate, and four herbicides, *viz.*, pendimethalin, glyphosate, alachlor and 2,4-D sodium salt were used to assess their toxicity at different concentrations on test *Trichoderma* isolates *in vitro* by using the poisoned food technique (Nene and Thapliyal, 1993). Radial growth of the test *Trichoderma* isolates were recorded after 48 h of incubation and per cent inhibition of growth was calculated using the following formula:

$$I = \frac{C - T}{C} \times 100$$ where

I – per cent inhibition
C – growth in unamended medium
T – growth in fungicide amended medium

Different concentrations of individual chemical were assessed in order to arrive at EC_{50} and EC_{90} values calculated using probit analysis programme, *viz.*, EPA Probit Analysis Programme in M STAT C.

RESULTS AND DISCUSSION

Both the isolates of *Trichoderma*, *viz.*, *T. harzianum* Th4 and *T. virens* Tv9 grew equally well and attained a radial growth of 9.0 cm after 48 h of incubation at 28±1°C on unamended PDA plates.

In agrochemicals amended medium when EC_{50} values were calculated based on inhibition per cent, all the agrochemicals showed varied inhibitory effect on radial growth (Tables 1 to 4). Variation also existed between the two test *Trichoderma* isolates in their sensitivity. Chemicals with EC_{50} values above the recommended field concentration were considered highly toxic and less than concentrations were considered as less toxic.

Toxicity of fungicides

When mean EC_{50} values of different fungicides were compared, benzimidazoles, *viz.*, carbendazim and benomyl were found highly toxic (EC_{50} 0.5 and 0.9 ppm and EC_{90} 3.6 and 5.4 ppm respectively) of all the fungicides (Table 1). Except azoxystrobin (mean EC_{50} 3491.1 ppm) all other systemic fungicides tested such as carboxin, metalaxyl, fosetyl Al, triazoles (hexaconazole, propiconazole and tricyclazole) and tridemorph were found highly toxic when their mean EC_{50} values were compared with respective recommended field concentrations. Among the contact fungicides wettable sulphur (inorganic S with mean EC_{50} 64542.7 ppm), Bordeaux mixture (EC_{50} 36365.4 ppm) and mancozeb (organic S with mean EC_{50} 3191.4 ppm) were found less toxic where as copper oxychloride (proprietory copper group), thiram (organic S), captan, chlorothalonil and dinocap were found highly toxic with mean EC_{50} values less than their recommended field concentrations. It may be noted here that all the three most commonly used seed dressing fungicides were found highly toxic to *Trichoderma*, *i.e.*, carbendazim, thiram and captan and hence requires cautious approach for their use in integrated disease management as seed dressing chemicals along with *Trichoderma*. When mean EC_{50} values over all the fungicides were compared for individual *Trichoderma* isolate, *T. virens* Tv9 (mean EC_{50} of 3957.8 ppm) was found more sensitive to fungicides than *T. harzianum* Th4 (mean EC_{50} of 8261.9 ppm). Except for propiconazole, tridemorph and copper oxychloride, *T. virens* Tv9 was found sensitive to all other test fungicides compared to *T. harzianum* Th4 which indicated relative tolerance of *T. harzianum* Th4 to fungicides.

Toxicity of insecticides

Among the eleven insecticides tested and compared for their mean EC_{50} values over both the *Tri-*

Table 1 : Toxicity of fungicides on isolates of *Trichoderma*.

Fungicide	Recommended field concentration. (ppm)	Concentration in ppm at 95 FL					
		EC_{50}			EC_{90}		
		Th 4	Tv 9	Mean	Th 4	Tv 9	Mean
Carbendazim	1000	0.42	0.59	0.5	1.13	6.13	3.6
Benomyl	1000	0.85	0.89	0.9	2.24	8.64	5.4
Carboxin	2000	420.00	224.50	322.3	2100.20	46831.60	24465.9
Metalaxyl	2000	421.70	286.60	354.2	948.80	1317.60	1133.2
Fosetyl Al	1500	950.50	487.80	719.2	2000.10	2878.50	2439.3
Hexaconazole	2000	61.20	13.60	37.4	3645.60	11254.10	7449.9
Propiconazole	1000	1.40	6.04	3.7	20.13	4380.30	2200.2
Tricyclazole	600	137.00	42.35	89.7	2803.40	4634.40	3718.9
Tridemorph	1000	3.10	5.75	4.4	124.70	148.00	136.4
Azoxystrobin	1000	3965.10	2873.00	3419.1	2484611.20	85393.30	1285002.3
Copper oxychloride	3000	258.00	310.30	284.2	11563.10	963.30	6263.2
Bordeaux mixture	10000	44692.65	28038.20	36365.4	226620.60	167596.20	197108.4
Wettable sulphur	2000	91284.10	37801.20	64542.7	731640.70	1511692.20	1121666.5
Mancozeb	2500	5676.30	706.50	3191.4	253052.20	452412.20	352732.2
Thiram	2500	614.90	312.10	483.5	1466.30	7832.60	4649.5
Captan	2000	172.60	87.60	130.1	1673.60	2660.40	2167.0
Chlorothalonil	2000	2.21	0.19	1.2	1220.40	1.90	611.2
Dinocap	1000	52.20	43.65	47.9	93224.00	18773.00	55998.5
Mean		8261.90	3957.80	6109.9	212039.90	128821.40	170430.6

choderma isolates, all the three test organophosphorous compounds such as quinalphos (EC_{50} 34.1 ppm), chlorpyriphos (EC_{50} 185.9 ppm) and dimethoate (EC_{50} 1036.6 ppm), and endosulfan, an organochlorine chemical (EC_{50} 975 ppm) were found highly toxic to *Trichoderma* isolates (Table 2). Carbamate fungicide carbofuran was found least toxic to *Trichoderma* isolates with mean EC_{50} as high as 66529 ppm. Other test insectides belonging to the new generation chemicals such as fipronil, imidachloprid, thiamethoxam, emamectin benzoate, indoxacarb and spinosad were found less toxic with mean EC_{50} values lower their recommended field concentrations. When comparisons were made between the two test isolates with EC_{50} values over all the insecticides tested, *T. virens* Tv9 was found highly sensitive (mean EC_{50} 13356.3 ppm) compared to *T. harzianum* Th4 (mean EC_{50} 18839.7 ppm). When individual comparisons are made, except for thiamethoxam for all other insecticides *T. virens* Tv9 was more sensitive compared to *T. harzianum* Th4.

Toxicity of fertilizers

As fertilizers are directly applied to the field either through broadcast method or furrow placement there by *Trichoderma* is likely to get direct exposure to these fertilizers, Hence, EC_{50} values were compared with ten times (20000 ppm) the concentration recommended for their foliar spray (2000 ppm). Three of the most commonly used fertilizers, *i. e.*, zinc sulphate (mean EC_{50} 1387.3 ppm), diammonium phosphate (7021.1 ppm) and urea (16811.7 ppm) were found highly toxic to *Trichoderma* isolates (Table 3). Remaining three test fertilizers studied, *i. e.*, muriate of potash, ammonium sulphate and potassium nitrate were found

Table 2 : Toxicity of insecticides on isolates of *Trichoderma*

Insecticide	Recommended field concentration. (ppm)	Concentration in ppm at 95 FL					
		EC_{50}			EC_{90}		
		Th4	Tv9	Mean	Th4	Tv9	Mean
Endosulfan	2000	1636.2	313.8	975.0	5383.7	2694.0	4038.9
Chlorpyrphos	2500	181.6	190.2	185.9	1977.8	2461.8	2219.8
Dimethoate	2000	1521.6	551.5	1036.6	10070.0	1584.1	5827.1
Quinalphos	2000	48.9	19.31	34.1	898.9	173.3	536.1
Fipronil	2000	8735.9	2330.1	5533.0	384591.4	49434.3	217012.9
Imidachloprid	250	8923.9	4944.2	6934.1	21072.6	59742.9	40407.8
Thiamethoxam	200	9568.8	50828.0	30198.4	38694.6	2347723.5	1193209.1
Emamectin benzoate	450	6891.9	5823.2	6357.6	686962.6	12433.8	349698.2
Indoxacarb	1000	13118.7	7313.2	10215.9	192225.5	20093.4	106159.5
Spinosad	400	85108.2	13049.0	49078.6	617755.5	976584.5	797170.0
Carbofuran	2000	71501.4	61556.6	66529.0	800705.6	8617881.0	4709293.3
Mean		18839.7	13356.3	16098.0	250939.8	1099164.2	675052.4

Table 3 : Toxicity of fertilizers on isolates of *Trichoderma*

Fertilizer	Recommended field spray concentration. (ppm)	Concentration in ppm at 95 FL					
		EC_{50}			EC_{90}		
		Th4	Tv9	Mean	Th4	Tv9	Mean
Zinc sulphate	2000	1843.6	931.0	1387.3	8247.8	2537.9	5392.9
Diammonium phosphate	2000	10946.0	3096.2	7021.1	41127.8	26924.5	34026.2
Urea	2000	17984.4	15638.9	16811.7	40510.7	184044.4	112277.6
Muriate of potash	2000	48576.7	46730.9	47653.8	101479.1	110338.6	105908.9
Ammonium sulphate	2000	50780.4	42660.2	46720.3	109040.9	243647.6	176344.3
Potassium nitrate	2000	58128.8	49096.9	53612.9	157870.3	209529.7	183700.0
Mean		31376.7	26359.0	28867.9	76379.4	129503.8	102941.7

less toxic. Isolate *T. virens* Tv9 was found more sensitive (mean EC_{50} 26359 ppm) to all the fertilizers tested compared to *T. harzianum* Th4 (31376.7 ppm) when mean EC_{50} values were compared over all the fertilizers and also individually.

Toxicity of herbicides

Evaluation of EC_{50} values of herbicides over both the *Trichoderma* isolates indicated that alachlor (157.5 ppm), pendemethalin (440.6 ppm) and glyphosate (1782 ppm) were highly toxic where as 2, 4 – D was less toxic (4613.4) when compared to respective recommended field concentrations (Table 4). Of both the isolates tested, *T. virens* Tv9 (mean EC_{50} 1139.5 ppm) was more sensitive than *T. harzianum* Th4 (2357.3 ppm) when comparisons were made over all the herbicides and also individually.

Table 4 : Toxicity of herbicides on isolates of *Trichoderma*.

Herbicide	Recommended field concentration. (ppm)	Concentration in ppm at 95 FL					
		EC_{50}			EC_{90}		
		Th4	Tv9	Mean	Th4	Tv9	Mean
Alachlor	6000	231.3	83.64	157.5	980.9	652.7	816.8
Pendimethalin	7000	770.4	110.8	440.6	4375.5	12591.2	8483.4
Glyphosate	6000	2343.7	1220.3	1782.0	23756.3	15712.0	19734.2
2,4-D Sodium salt	4000	6083.6	3143.1	4613.4	64730.5	120840.0	92785.3
Mean		2357.3	1139.5	1748.4	23460.8	37448.9	30454.9

The present study revealed higher sensitivity of *T. virens* Tv9 isolated from citrus orchard where in the isolate was less exposed to several of the xenobiotics available for plant protection. In other words, *T. harzianum* Th4 obtained from cotton ecosystem was more tolerant to agrochemical toxicity owing to its continuous exposure to applied xenobiotics. Hence, from the present study it may be interpreted that agrochemical tolerant and effective *Trichoderma* isolates can be better isolated from the cropping systems that are continuously exposed to different agrochemicals such as cotton for further utilization in IDM strategies.

Inhibitory effect of different agrochemicals on *Trichoderma* spp. was reported by Akbari and Parakhia (2001), Srinivasulu *et al.* (2002), Vijayaraghavan and Abraham (2004), Upadhyay *et al.* (2004), Tiwari *et al.* (2004), Rai Ajay Kumar *et al.* (2005) and Pandey *et al.* (2006) along with isolate variation. Dubey and Patel (2001) reported tolerance levels of *T. viride* and *Gliocladium virens* to fungicides. However, a great deal of work done was based on arbitrary concentrations that were less than the recommended field concentrations. In the present study calculation of EC_{50} and EC_{90} values of test agrochemicals against two different *Trichoderma* isolates obtained from two different agroecosystems revealed the range of sensitivity to these test agrochemicals. Further, comparisons made with the recommended field concentrations indicated possibility of agrochemicals adversely affecting efficacy of applied *Trichoderma* isolates.

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