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Sensitivity of *Trichoderma* Isolates to Selected Insecticides *in vitro*

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ABSTRACT

Laboratory experiment was conducted to assess the Sensitivity of *Trichoderma* isolates to eleven insecticides at concentration recommended for field use by following poisoned food technique and spore germination technique. The test *Trichoderma* isolates were found highly sensitive to organochlorine compound endosulfan and organophosphates namely chlorpyriphos, quinalphos and dimethoate and insensitivity to new generation insecticides like imidacloprid, thiamethoxam, emamectin benzoate and spinosad in assimilative phase, while on spore phase all the eleven insecticides were found toxic showing varied degree of inhibition. Based on radial growth and spore germination inhibition all the eleven insecticides were categorized into dangerous, cautious and safe groups. All the three insecticides belonging to organophosphates namely chlorpyriphos, quinalphos and dimethoate were found place in dangerous category, while endosulfan, thiamethoxam, emamectin benzoate, fipronil and spinosad were found place in cautious group. Only indoxacarb and imidacloprid were found safe to the test *Trichoderma* isolates.

Key words : Categorization, Insecticides, Sensitivity, *Trichoderma*.

Soil borne plant pathogens 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 (Papavizas, 1985; and Mukhopadhyay, 1987). *Trichoderma* spp. 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).

Trichoderma spp. are used either as seed treatment or soil application. In both the cases, the antagonist has been continuously exposed to different insecticides applied to the field either in soil or as foliar sprays. Insecticides sprayed aerially reaches the soil (by means of air currents or are washed off the plant surface due to rain) and are likely to influence the efficacy of native or applied biocontrol agents like *Trichoderma*. Hence it is necessary to know *Trichoderma* sensitivity to insecticides before applying in the field so that precautionary measures can be taken to prevent decrease in the antagonist's efficacy. Variation in the tolerance of antagonists to several agrochemicals reported earlier (Reshmy Vijayaraghavan and Koshy Abraham, 2004 and Madhavi *et al* , 2008) were based on arbitrary concentrations that were less than the field concentrations.

Hence, the present investigation was conducted to evaluate the sensitivity of two isolates of *Trichoderma* spp. *viz., T. harzianum* (isolated from cotton cropping system) and *T. virens* (isolated from citrus orchard) to selected insecticides at field concentration.

MATERIAL AND METHODS

In the present investigation eleven insecticides *viz.,* endosulfan, chlorpyrifos, quinalphos, dimethoate, indoxacarb, carbofuran, imidacloprid, fipronil, thiamethoxam, emamectin benzoate and spinosad were used to assess the sensitivity of *Trichoderma* isolates by using the poisoned food technique (Nene and Thapliyal, 1993) and slide germination technique (Montgomery and Moore, 1938). Radial growth or spore germination of the test *Trichoderma* isolates were recorded after 48 h of incubation and per cent inhibition of growth or spore germination over control (unamended medium) was calculated using the following formula:

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

Where,

I – per cent inhibition

C – growth spore germination^{-1} in unamended medium

T - growth spore germination^{-1} in fungicide amended medium

Table 1. Effect of insecticides on the radial growth of *Trichoderma spp.*

S. No.	Insecticides	Conc.	Radial growth (cm)			
			T. harzianum		*T. virens*	
			Day_1	Day_2	Day_1	Day_2
1.	Endosulfan	0.2%	2.1 (1.8)[f]	3.7 (2.2)[b]	0.9 (1.4)[e]	1.1 (1.4)[e]
2.	Chlorpyriphos	0.25%	0.0 (1.0)[h]	0.0 (1.0)[d]	0.0 (1.0)[f]	0.0 (1.0)[f]
3.	Quinalphos	0.2%	0.0 (1.0)[h]	0.0 (1.0)[d]	0.0 (1.0)[f]	0.0 (1.0)[f]
4.	Dimethoate	0.2%	1.2 (1.5)[g]	3.1 (2.0)[c]	0.0 (1.0)[f]	0.0 (1.0)[f]
5.	Indoxacarb	0.1%	4.9 (2.4)[b]	9.0 (3.2)[a]	3.5 (2.1)[c]	8.5 (3.1)[b]
6.	Carbofuran	0.2%	3.6 (2.1)[e]	9.0 (3.2)[a]	2.3 (1.8)[d]	7.5 (2.9)[c]
7.	Imidacloprid	0.025%	4.3 (2.3)[c]	9.0 (3.2)[a]	4.6 (2.4)[a]	9.0 (3.2)[a]
8.	Fipronil	0.2%	3.4 (2.1)[e]	9.0 (3.2)[a]	2.2 (1.8)[d]	7.1 (2.8)[d]
9.	Thiamethoxam	0.02%	5.1 (2.5)[a]	9.0 (3.2)[a]	3.4 (2.1)[c]	9.0 (3.2)[a]
10.	Emamectin benzoate	0.045%	3.5 (2.1)[e]	9.0 (3.2)[a]	3.7 (2.2)[b]	9.0 (3.2)[a]
11.	Spinosad	0.04%	3.9 (2.2)[d]	9.0 (3.2)[a]	3.6 (2.1)[c]	9.0 (3.2)[a]
12.	Check		4.2 (2.3)[c]	9.0 (3.2)[a]	3.8 (2.2)[b]	9.0 (3.2)[a]
	CV (%)		1.5	1.1	1.6	1.1
	CD (P=0.01)		0.06	0.06	0.06	0.06

*Each treatment replicated thrice
*Figures in parentheses are square root transformed values
* Figures with similar alphabets do not differ significantly

RESULTS AND DISCUSSION

The result indicated that in the insecticide unamended medium *i.e.,* control plate *T.harzianum* and *T.virens* showed a growth of 4.2cm and 3.8cm after 24 hours of incubation and covered the entire petriplate (9.0 cm) in 48 hours of incubation. When the medium was amended with test insecticides, significant differences were observed in the growth and in turn on the sensitivity of *Trichoderma* isolates under study when compared to check plates.

Eight out of eleven insecticides tested, *viz.,* endosulfan, chlorpyrifos, quinalphos, dimethoate, carbofuran, fipronil, emamectin benzoate and spinosad were found toxic to *T harzianum* isolate while with the remaining three test insecticides, *i.e*., indoxacarb (4.9 cm), imidacloprid (4.3 cm) and thiamethoxam (5.1 cm), *T harzianum* was found insensitive when compared to check (4.2 cm) after 24 hours of inoculation. Isolate of *T virens* was found sensitive to ten out of eleven insecticides tested, *viz.,* endosulfan, chlorpyriphos, quinalphos, dimethoate, indoxacarb, carbofuran, fipronil, thiamethoxam, emamectin benzoate and spinosad and insensitive to only imidacloprid (4.6 cm) on 1st day of incubation. Observations on the 2nd of incubation indicated that *T harzianum* was found sensitive to endosulfan, chlorpyriphos, quinalphos and dimethoate and *T virens* was found sensitive to seven out of eleven insecticides tested, *viz.,* endosulfan, chlorpyriphos, quinalphos, dimethoate, indoxacarb, carbofuran and fipronil (Table1).

The mean per cent inhibition calculated over both the isolates indicated that *T harzianum* and *T virens* vary in their sensitivity towards selected eleven insecticides. Chlorpyriphos and quinalphos showed complete inhibition on both the test isolates at both the days of incubation, which differed significantly with all other insecticides studied, followed by dimethoate (72.0 per cent inhibition), endosulfan (57.4 per cent inhibition), carbofuran (11.9 per cent inhibition), fipronil (14.0 per cent inhibition) and indoxacarb(7.0 per cent inhibition) (Table 2).

Dimethoate showed selective inhibitory effect on both the test isolates - *T harzianum* was moderately inhibited (65.5 per cent inhibition) and *T virens* was completely inhibited (100 per cent inhibition). Similarly, *T harzianum* was insensitive to carbofuran while *T virens* was inhibited (16.3 per cent inhibition).

When the mean per cent inhibition of individual isolates, *i.e., T harzianum* and *T virens* were compared over all the insecticides evaluated, *T harzianum* was found less sensitive (29.5 per cent inhibition) compared to *T virens* (39.1 per cent inhibition) with significant difference between them.

Interaction effect indicated that *T virens* was comparatively and significantly more sensitive to endosulfan (87.7 per cent inhibition in *T virens* and 59.2 per cent inhibition in *T harzianum*), dimethoate (100.0 per cent inhibition in *T virens* and 65.5 per cent inhibition in *T harzianum*), indoxacarb (6.0 per cent inhibition in *T virens* and 0.0 per cent inhibition in *T harzianum*), carbofuran (16.3 per cent inhibition in *T virens* and 0.0 per cent inhibition in *T harzianum*) and fipronil (21.1 per cent inhibition in *T virens* and 0.0 per cent inhibition in *T harzianum*).

Inhibition of spore germination:

Eleven insecticides were assessed for their impact on spore germination of *T harzianum* and *T virens* using slide germination technique. Observations were taken on number of spores germinated per microscopic field in treatment slides and compared with spore germination in check slides.

When the mean per cent inhibition of spore germination was calculated over both the isolates, *i.e., T harzianum* and *T virens* and compared, the result indicated that among the eleven selected insecticides, two insecticides *viz.,* chlorpyrifos and quinalphos showed 100.0 per cent spore inhibition and differed significantly with the rest of the nine insecticides tested, *i.e.,* emamectin benzoate (77.8 per cent inhibition), dimethoate (75.0 per cent inhibition), thiamethoxam (60.7 per cent inhibition), fipronil (56.5 per cent inhibition), carbofuran (53.8 per cent inhibition), endosulfan (50.0 per cent inhibition), spinosad (49.1 per cent inhibition), imidacloprid (41.8 per cent inhibition) and indoxacarb (38.3 per cent inhibition) (Table 3).

When the mean per cent inhibition of *T harzianum* and *T virens* were compared over all the insecticides evaluated, both the isolates were sensitive with more than 60.0 per cent inhibition in spore germination, though significant difference existed between the two isolates with *T virens* found more sensitive (67.0 per cent inhibition) compared to *T harzianum* (60.8 per cent inhibition).

Interaction effect indicated that *T virens* was comparatively and significantly more sensitive to endosulfan (53.8 per cent inhibition in *T virens* and 46.2 per cent inhibition in *T harzianum*), dimethoate (82.2 per cent inhibition in *T virens* and 67.9 per cent inhibition in *T harzianum*), indoxacarb (39.4 per cent inhibition in *T virens* and 37.2 per cent inhibition in *T harzianum*), imidacloprid (45.0 per

Table 2. Effect of insecticides on the radial growth of *Trichoderma*

S. No.	Insecticides	Conc.	Per cent inhibition in radial growth		
			T.harzianum	*T.virens*	Mean
1.	Endosulfan	0.2%	59.2 (45.3)	87.7 (69.4)	73.4 (57.4)[c]
2.	Chlorpyriphos	0.25%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
3.	Quinalphos	0.2%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
4.	Dimethoate	0.2%	65.5 (54.0)	100.0 (90.0)	82.7 (72.0)[b]
5.	Indoxacarb	0.1%	0.0 (0.0)	6.0 (14.0)	3.0 (7.0)[e]
6.	Carbofuran	0.2%	0.0 (0.0)	16.3 (23.8)	8.2 (11.9)[d]
7.	Imidacloprid	0.025%	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[f]
8.	Fipronil	0.2%	0.0 (0.0)	21.1 (27.3)	10.5 (14.0)[d]
9.	Thiamethoxam	0.02%	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[f]
10.	Emamectin benzoate	0.045%	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[f]
11.	Spinosad	0.04%	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[f]
12.	Check		29.5 (25.4)[b]	39.1 (36.7)[a]	
	CV (%)			6.3	
	CD (P=0.01)		Insecticide 3.0	Isolate 1.3	Insecticide X Isolate 4.3

*Each treatment replicated thrice
*Figures in parentheses are square root transformed values
* Figures with similar alphabets do not differ significantly

cent inhibition in *T virens* and 38.5 per cent inhibition in *T harzianum*), fipronil (64.2 per cent inhibition in *T virens* and 48.7 per cent inhibition in *T harzianum*), thiamethoxam (63.8 per cent inhibition in *T virens* and 57.5 per cent inhibition in *T harzianum*), emamectin benzoate (83.5 per cent inhibition in *T virens* and 72 per cent inhibition in *T harzianum*) and spinosad (55.1 per cent inhibition in *T virens* and 43.1 per cent inhibition in *T harzianum*). On the other hand, *T harzianum* was found more sensitive to only carbofuran (57.7 per cent inhibition in *T harzianum* and 49.9 per cent inhibition in *T virens*).

This indicated that test *Trichoderma* isolates were highly sensitive to organochlorines (endosulfan) and organo phosphates (chlorpyrifos, quinalphos and dimethoate). Sushir and Pandey (2001) reported toxic effect of endosulfan on the radial growth of *T.harzianum*. Similar observation was also made by Reshmy Vijayaraghavan and Koshy Abraham (2004). The test *Trichoderma* isolates showed insensitivity to new generation insecticides like imidacloprid, thiamethoxam, emamectin benzoate and spinosad indicating their highly compatible nature with these insecticides in

Table 3. Effect of fungicides on *Trichoderma* - per cent inhibition in spore germination.

S. No.	Insecticides	Conc.	*T.harzianum*	*T.virens*	Mean
1.	Endosulfan	0.2%	46.2 (42.8)	53.8 (47.2)	50.0 (45.0)^{cd}
2.	Chlorpyriphos	0.25%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)^{a}
3.	Quinalphos	0.2%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)^{a}
4.	Dimethoate	0.2%	67.9 (55.5)	82.2 (65.0)	75.1 (60.2)^{b}
5.	Indoxacarb	0.1%	37.2 (37.5)	39.4 (38.9)	38.3 (38.2)^{ef}
6.	Carbofuran	0.2%	57.7 (49.4)	49.9 (44.9)	53.8 (47.2)^{cd}
7.	Imidacloprid	0.025%	38.5 (38.3)	45.0 (42.1)	41.8 (40.2)^{def}
8.	Fipronil	0.2%	48.7 (44.3)	64.2 (53.3)	56.5 (48.8)^{cd}
9.	Thiamethoxam	0.02%	57.5 (49.3)	63.8 (53.0)	60.7 (51.1)^{c}
10.	Emamectin benzoate	0.045%	72.0 (58.0)	83.5 (66.0)	77.8 (62.0)^{b}
11.	Spinosad	0.04%	43.1 (41.0)	55.1 (47.9)	49.1 (44.5)^{cde}
12.	Check		60.8 (54.2)^{b}	67.0 (58.0)^{a}	
	CV (%)			4.2	
	CD (P=0.01)		Insecticide 4.7	Isolate 2.0	Insecticide X Isolate 6.7

*Each treatment replicated thrice
*Figures in parentheses are square root transformed values
* Figures with similar alphabets do not differ significantly

assimilative phase. Prasanna *et al.* (2002) reported compatibility of *T.harzianum* to thiamethoxam even up to 1% concentration. Lal and Maharshi (2007) reported moderate compatibility of *Trichoderma* spp. with imidacloprid at 500 ppm concentration. When spore phase was evaluated, all the eleven insecticides were found toxic, showing varied degree of inhibition (mean spore inhibition from 38.3 to 100%) (Table 3).

The result clearly showed that *T harzianum*, isolate from cotton cropping system where in pesticide usage is maximum was found less sensitive to the insecticides compared to *T virens,* the isolate from citrus orchard where in insecticide usage is less. All the insecticides were used in the present study were categorized as "Dangerous", "Cautious" and "Safe" based on the per cent inhibition in radial growth and spore germination of *Trichoderma* isolates (Table 4). Among eleven insecticides evaluated, all the three insecticides belonging to organophosphates *viz.,* chlorpyriphos, quinalphos and dimethoate were in the category of I to V and designated as **"**dangerous**".**

The 'cautious' group of insecticides included endosulfan, thiamethoxam and emamectin benzoate and only indoxacarb and imidacloprid were found safe to *Trichoderma* isolates.

Table 4. Categorization of insecticides based on per cent inhibition in the radial growth and spore germination of *Trichoderma*.

S. No	Spore germination	Radial growth	Category	Group	
1.	100	100	I		
2.	100	> 50	II	Dangerous	chlorpyriphos, quinalphos, dimethoate
3.	100	< 50	III		
4.	> 50	100	IV		
5.	< 50	100	V	Cautious	endosulfan, carbofuran, thiamethoxam, emamectin benzoate, fipronil, spinosad
6.	> 50	> 50	VI		
7.	> 50	< 50	VII		
8.	< 50	> 50	VIII		
9.	< 50	< 50	IX	Safe	imidacloprid, indoxacarb
10.	0	0	X		

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