The Andhra Agric. J 59(1): 77-82, 2012

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

Ranganathswamy M, Patibanda A K, Krishna Murthy K V M and Prasuna Rani P
Department of Plant Pathology, Agricultural College, Bapatla-522 101, Andhra Pradesh.

ABSTRACT

Eighteen selected fungicides were categorized as dangerous, cautious and safe to *Trichoderma* based on *in vitro* senstitivity of *T. harzianum* and *T. virens*. Observations on radial growth and spore germination indicated that Bordeaux mixture, mancozeb, thiram, captan, carbendazim, benomyl, carboxin, metalaxyl, propiconazole, hexaconazole, tricyclozole, tridemorph, fosetyl-Al, chlorothalonil and azoxystrobin were dangerous to *Trichoderma* spp with 100 per cent inhibition of either radial growth or spore germination or both. While none of the test fungicides were found safe to *Trichoderma*, cautious category included copper oxychloride, dinocap and wettable sulphur.

Key words : Categorization, Fungicides, Sensitivity, *Trichoderma*

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 and 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).

Species of *Trichoderma* are being used either as seed treatment or soil application. In both the cases, the antagonist has been continuously exposed to different fungicides applied to the field either in soil or as foliar sprays. Fungicide 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 Trichoderma sensitivity to fungicides in order to use in the Integrated Disease Management systems (Singh *et al*. 1995). Variations in tolerance of *Trichoderma* isolates to several fungicides reported earlier (Pandey and Upadhyay, 1998; Reshmy Vijayaraghavan and Koshy Abraham, 2004) 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 fungicides at field concentration.

MATERIAL AND METHODS

In the present investigation eight contact fungicides, *viz.,* copper oxychloride, Bordeaux mixture, wettable sulphur, mancozeb, thiram, captan, chlorothalonil and dinocap and ten selective systemic fungicides, *viz.,* carbendazim, benomyl, carboxin, metalaxyl, propiconazole, hexaconazole, tricyclazole, tridemorph, fosetyl-Al and azoxystrobin, were used to assess the *in vitro* sensitivity of *Trichoderma* isolates by using 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:

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

I - Per cent inhibition
C - growth / spore germination in unamended medium
T - growth / spore germination in fungicide amended medium

RESULTS AND DISCUSSION

Both the isolates of *Trichoderma*, *viz*., *T harzianum* and *T virens* grew equally well and attained a radial growth of 3.7 cm and 9.0 cm after

Table 1. Effect of fungicides on the radial growth of *Trichoderma.*

S. No.	Fungicides	Conc.	Radial growth in cm			
			T. harzianum		*T. virens*	
			Day1	Day2	Day1	Day2
1	Copper oxy chloride	0.3%	1.0 (1.4)^{f}	2.5 (1.9)^{dc}	0.6 (1.3)^{g}	2.0 (1.7)^{d}
2.	Bordeaux mixture	1%	3.4 (2.1)^{c}	7.8 (3.0)^{b}	2.5 (1.9)^{c}	6.5 (2.7)^{b}
3.	Wettable sulphur	0.2%	4.1 (2.3)^{a}	9.0 (3.2)^{a}	3.3 (2.1)^{b}	8.7 (3.1)^{a}
4.	Mancozeb	0.25%	2.5 (1.9)^{d}	6.2 (2.7)^{c}	1.9 (1.7)^{e}	4.1 (2.3)^{c}
5.	Thiram	0.25%	0.0 (1.0)^{h}	1.0 (1.4)^{g}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
6.	Captan	0.2%	0.6 (1.3)^{g}	1.8 (1.7)^{f}	0.6 (1.3)^{g}	1.4 (1.5)^{e}
7.	Dinocap	0.1%	1.4 (1.5)^{e}	3.1 (2.0)^{d}	0.9 (1.4)^{f}	2.0 (1.7)^{d}
8.	Chlorothalonil	0.2%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
9.	Carbendazim	0.1%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
10.	Benomyl	0.1%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
11.	Carboxin	0.2%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
12.	Metalaxyl	0.2%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.7 (1.3)^{g}	1.1 (1.4)^{e}
13.	Propiconazole	0.1%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
14.	Hexaconazole	0.2%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
15.	Tricyclazole	0.06%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
16.	Tridemorph	0.1%	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{h}	0.0 (1.0)^{f}
17.	Fosetyl-Al	0.15%	1.1 (1.4)^{f}	2.4 (1.8)^{ef}	0.8 (1.3)^{g}	1.4 (1.5)^{e}
18.	Azoxystrobin	0.1%	2.5 (1.9)^{d}	5.6 (2.6)^{c}	2.2 (1.8)^{d}	6.1 (2.7)^{b}
19.	Check		3.7 (2.2)^{b}	9.0 (3.2)^{a}	3.7 (2.2)^{a}	9.0 (3.2)^{a}
	C V (%)		1.6	2.1	1.7	2.3
	CD (P=0.01)		0.06	0.10	0.06	0.10

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

Table 2. Effect of fungicides on *Trichoderma* radial growth - per cent inhibition

S. No.	Fungicides	Conc.	*T.harzianum*	*T.virens*	Mean
1.	Copper oxy chloride	0.3%	72.2 (58.2)	77.7 (61.8)	75 (60.0)[e]
2.	Bordeaux mixture	1%	13.6 (21.6)	27.7 (31.7)	20.6 (26.7)[h]
3.	Wettable sulphur	0.2%	0.0 (0.0)	4.4 (9.0)	2.2 (4.5)[i]
4.	Mancozeb	0.25%	31.5 (34.1)	54.5 (47.6)	43.0 (40.8)[f]
5.	Thiram	0.25%	88.8 (70.4)	100.0 (90.0)	94.4 (80.2)[b]
6.	Captan	0.2%	80.4 (63.7)	84.8 (67.0)	82.6 (65.3)[c]
7.	Dinocap	0.1%	65.5 (54.1)	77.3 (61.6)	71.4 (57.8)[e]
8.	Chlorothalonil	0.2%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
9.	Carbendazim	0.1%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
10.	Benomyl	0.1%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
11.	Carboxin	0.2%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
12.	Metalaxyl	0.2%	100.0 (90.0)	88.1 (69.8)	94.0 (79.8)[b]
13.	Propiconazole	0.1%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
14.	Hexaconazole	0.2%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
15.	Tricyclazole	0.06%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
16.	Tridemorph	0.1%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
17.	Fosetyl-Al	0.15%	74.0 (59.3)	84.0 (66.4)	79.0 (62.8)[d]
18.	Azoxystrobin	0.1%	37.7 (37.9)	31.8 (34.3)	35.0 (36.1)[g]
	Mean		75.8 (69.1)[b]	79.5 (72.3)[a]	
	C V (%)			2.3	
	CD (P=0.01)		Fungicides 2.3	Isolate 0.7	Fungicide X Isolate 3.3

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

24 and 48 h of incubation at 28±1°C respectively on control PDA plates (Table 1). Similarly, spore germination was on par in both the isolates with 100 per cent germination by 48th h of incubation in fungicide un-amended potato dextrose broth.

In fungicide amended medium, all the fungicides showed inhibitory effect either on radial growth or spore germination or both. Variation existed between *Trichoderma* isolates in their sensitivity to different fungicides, between the growth stages of the same test fungus, *i. e.,* assimilative phase (radial growth) and spore phase (spore germination) and among different fungicides in their toxicity to *Trichoderma* isolates.

Variation between isolates of *Trichoderma*: Observations made on the radial growth of *Trichoderma* indicated significant variation in the sensitivity of *Trichoderma* isolates to fungicides or toxicity of fungicides towards *Trichoderma* isolates. When observations were recorded on radial growth for two consecutive days except in Bordeaux mixture, copper oxychloride, wettable sulphur, mancozeb, captan, fosetyl-Al, azoxystrobin and dinocap amended plates, in all other plates the growth was completely inhibited within 24 h after inoculation. Further, all the fungicides showed significant reduction in the growth of Trichoderma isolates on 1st and 2nd day of observations except in wettable sulphur where in the growth was on par with unamended control. When mean inhibitory per cent in the radial growth of *Trichoderma* isolates was analyzed, cotton isolate *T. harzianum* was found less sensitive (75.8% inhibition) compared to citrus isolate *T. virens* (79.5%) (Table 2). Similar result was obtained with spore germination where in *T. harzianum* was less sensitive (94%) compared to *T. virens* (97%) (Table 3). This difference in per cent inhibition of radial growth was due to more sensitivity of *T. virens* to seven out of eight contact fungicides, *viz*., Bordeaux mixture, copper oxychloride, wettable sulphur, thiram, captan and dinocap and also to one systemic fungicide Fosetyl Al. Compared to *T. virens*, sensitivity of *T. harzianum* was higher only with respect to metalaxyl. Reports on inhibitory effect of different fungicides were reported by Sharma and Mishra (1995), Mondal et al. (1995), Karpagavalli (1997), Reshmy Vijayaraghavan and Koshy Abraham (2004) and Pandey *et al.* (2006).

Though both the *Trichoderma* isolates were equally sensitive to fifteen of the eighteen test fungicides in spore germination (100% inhibition), copper oxychloride (80%), wettable sulphur (81%) and dinocap (82%) had more effect on *T. virens* than on *T. harzianum* (63, 64 and 66% respectively). Relatively high sensitivity of *T. virens* may be due to the fact that the isolate was obtained from citrus orchard which is less exposed to the fungicidal application. In other words, *T. harzianum* was less sensitive as it was isolated from cotton where in the fungicide usage is more.

Variation between growth stages:

Sensitivity of *Trichoderma* isolates to selected eighteen fungicides was found to be more in spore phase compared to assimilative phase (radial growth) as fifteen out of eighteen fungicides could completely inhibit (100%) spore germination. With the remaining three fungicides, *viz*., copper oxychloride, wettable sulphur and dinocap, the inhibition in spore germination varied between 72 to 74 per cent. The assimilative phase is completely sensitive (100% inhibition) to only eight out of eighteen fungicides. With the remaining ten fungicides, inhibition in radial growth varied from 2.2 in wettable sulphur to 94.4 in thiram amended medium. Variation in the effect of fungicides on different growth stages of *Trichoderma* was maximum with Bordeaux mixture, wettable sulphur, azoxystrobin and mancozeb which were found to be less toxic to assimilative phase but highly toxic to spore phase.

Variation in fungicide toxicity:

Among the systemic chemicals, azoxystrobin was found least inhibitory (35% in growth) to *Trichoderma* isolates followed by 79 per cent with fosetyl Al and 94 per cent with metalaxyl. All other systemic fungicides were 100 per cent inhibitory. It may be noted here that both the benzimidazole group fungicides (benomyl and carbendazim), all the three triazole fungicides tested were 100 per cent inhibitory. Among the contact group of fungicides, only chlorothalonil showed 100 per cent inhibition in the radial growth of *Trichoderma* isolates while others gave 2.2 to 94 per cent inhibition in radial growth. Bordeaux mixture (20.6 and 100%), wettable sulphur (2.2 and 72.8%), mancozeb (43 and 10%) and azoxystrobin (35 and 100%) showed variation in their toxicity to assimilative and spore phases.

With in a fungicidal group variation existed in terms of toxicity to *Trichoderma* spp. When two copper fungicides were tested, viz., Bordeaux mixture (preparatory) and copper oxychloride (proprietary), Bordeaux mixture showed more inhibitory effect on spore germination than on radial growth. On the other hand, copper oxychloride showed more inhibitory effect on radial growth than on spore germination. Similar variation also existed

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

S. No.	Fungicides	Conc.	*T.harzianum*	*T.virens*	Mean
1.	Copper oxy chloride	0.3%	63.0 (7.9)	80.0 (8.9)	72 (8.4)[b]
2.	Bordeaux mixture	1%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
3.	Wettable sulphur	0.2%	64.5 (8.0)	81.0 (9.0)	72.8 (8.5)[b]
4.	Mancozeb	0.25%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
5.	Thiram	0.25%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
6.	Captan	0.2%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
7.	Dinocap	0.1%	66.0 (8.1)	82.0 (9.1)	74.0 (8.6)[a]
8.	Chlorothalonil	0.2%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
9.	Carbendazim	0.1%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
10.	Benomyl	0.1%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
11.	Carboxin	0.2%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
12.	Metalaxyl	0.2%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
13.	Propiconazole	0.1%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
14.	Hexaconazole	0.2%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
15.	Tricyclazole	0.06%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
16.	Tridemorph	0.1%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
17.	Fosetyl-Al	0.15%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
18.	Azoxystrobin	0.1%	100.0 (10.0)	100.0 (10.0)	100.0 (10.0)[a]
	Mean		94.0 (9.7)[b]	97.0 (9.8)[a]	
	C V (%)			1.27	
	CD (P=0.01)		Fungicides 0.23	Isolate 0.079	Fungicide X Isolate 0. 33

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

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

S. No	Spore germination	Radial growth	Category	Group	Fungicides
1.	100	100	I		Bordeaux mixture, mancozeb,thiram, captan,
2.	100	> 50	II		carbendazim, benomyl, carboxin, metalaxyl,
3.	100	< 50	III	Dangerous	propiconazole, hexaconazole, tricyclazole,
4.	> 50	100	IV		tridemorph, fosetyl-Al, chlorothalonil,
5.	< 50	100	V		azoxystrobin.
6.	> 50	> 50	VI	Cautious	copperoxychloride, dinocap, wettable sulphur
7.	> 50	< 50	VII		
8.	< 50	> 50	VIII		
9.	< 50	< 50	IX	Safe	———
10.	0	0	X		

with sulphur group. Wettable sulphur (inorganic S compound) was found least (2.2%) inhibitory to radial growth and more inhibitory to spore germination. Similar observations were also made with organic S compounds. Thiram was found more toxic than mancozeb to radial growth. However, both had 100 per cent inhibitory effect on spore germination.

Based on the results obtained, all the test fungicides were grouped as 'dangerous', 'cautious' and 'safe' and presented in Table 5. Bordeaux mixture, mancozeb, thiram, captan, carbendazim, benomyl, carboxin, metalaxyl, propiconazole, hexaconazole, tricyclozole, tridemorph, fosetyl Al, chlorothalonil and azoxystrobin were dangerous to *Trichoderma* spp with 100 per cent inhibition of either radial growth or spore germination or both. While none of the test fungicides were found safe to *Trichoderma*, copper oxychloride, dinocap and wettable sulphur were in cautious category.

LITERATURE CITED

Elad Y, Chet I and Katan I 1980 *Trichoderma harzianum* a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology* 70: 119-121.

Karpagavalli S 1997 Effect of different fungicides on the growth of *Trichoderma*. *Indian Journal of Plant protection* 25(1): 82-83.

Mondal G, Srivastava K D and Aggarwal R 1995 Antagonistic effect of *Trichoderma* spp on *Ustilago segetum* var *tritici* and their compatibility with fungicides and biocides. *Indian Phytopathology* 48: 466-470.

Montgomery H B S and Moore M H 1938 A laboratory method for testing the toxicity of protective fungicides. *Journal of Pomology and Horticulture Science* 15: 253-266.

Mukhopadhyay A N 1987 Biological control of soil borne plant pathogens by *Trichoderma* spp. *Indian Journal of Mycology and Plant Pathology* 17: 1-10.

Nene Y L and Thapliyal P N 1993 Fungicides in plant disease control 3rd ed. Oxford and IBH Publishing Co.Pv. Ltd. 691pp.

Pandey K K, pandey P K amd Mishra K K 2006 Bioefficacy of fungicides against different fungal bioagents for tolerance level and fungistatic behaviour. *Indian Phytopathology* 59: 68-71.

Pandey K K and Upadhyay J P1998 Sensitivity of different fungicides to *Fusarium udum, Trichoderma harzianum* and *Trichoderma viride* for integrated approach of disease management. *Vegetable Science* 25(1): 89-92.

Papavizas G C 1985 *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathology* 23: 23-54.

Reshmy Vijayaraghavan and Koshy Abraham 2004 Compatibility of biocontrol agents with pesticides and fertilizers used in black pepper gardens. *Journal of Mycology and Plant Pathology* 34(2): 506-510.

Sharma S D and Mishra A 1995 Tolerance of *Trichoderma harzianum* to agrochemicals. *Indian journal of Mycology and Plant Pathology* 25: 129-130

Singh R S, Jindal A, Singh D and Singh T 1995 Selection of *Trichoderma* isolates against common fungicides for their use in integrated plant disease management. *Indian Journal of Mycology and Plant Pathology* 25(2): 127-128.

(Received on 21.02.2011 and revised on 08.09.2011)