The Andhra Agric. J 61(4):857-863, 2014

Effect of Selected Agrochemicals on Radial Growth of *Trichoderma harzianum* (Th_4) *in Vitro*

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ABSTRACT

Commonly used agrochemicals at recommended field concentrations were evaluated for their toxicity to *Trichoderma harzianum* (Th_4) obtained from cotton ecosystem. Among fungicides, carbendazim showed higher toxicity followed by captan, metalaxyl, mancozeb and copper oxychloride. Chlorpyriphos was found more toxic followed by quizolofop ethyl among insecticides. Zinc sulphate followed by urea and diammonium phosphate was found to be the most toxic fertilizer. Imidacloprid, imazethapyr, single super phosphate, magnesium sulphate, muriate of potash and gypsum showed lesser inhibition on poisoned food and hence are likely to be compatible with *T. harzianum* (Th_4).

Key words : Insecticides, Fertilizers, Fungicides,Herbicides, *Trichoderma*, Toxicity.

Most of the agricultural crops are infected by soil borne plant pathogens, which primarily attack the vulnerable seeds or seedlings causing serious losses. Several fungi such as *Pythium, Phytophthora, Botrytis, Rhizoctonia, Sclerotium* and *Fusarium* are widely distributed in soil and effect various crops of economic importance (Chet *et al*., 1997).

Chemical means of managing the disease caused by these pathogens is not practicable owing to high cost of chemicals and environmental pollution. Biological control is a risk free method and results in enhancement of resident antagonists. Biological control using *Trichoderma* offers a novel approach when applied alone or in combination with other management practices (Papavizas, 1985).

Trichoderma spp. are the most common soil inhabitants effective in suppressing soil borne plant pathogens ((Papavizas, 1985) and offers an ecofriendly approach for protection of many crop plants against a wide range of soil borne plant pathogens (Pan and Jash, 2004). Species of *Trichoderma* are being used either through seed treatment or soil application to manage several soil borne plant diseases. When applied in the field, the antagonist is exposed to different agrochemicals such as fungicides, insecticides, fertilizers and herbicides that are used in crop management. Agrochemicals are likely to influence the development and efficacy of native or applied *Trichoderma*. The present investigation is aimed at evaluating the toxicity of selected agrochemicals at field concentrations on *T. harzianum* Th_4.

MATERIAL AND METHODS

Trichoderma harzianum (Th_4) obtained from cotton ecosystem, which was maintained in the Department of Plant Pathology, Agricultural College, Bapatla was used in the present investigation. Five fungicides *viz*., copper oxychloride, captan, mancozeb, carbendazim and metalaxyl; three insecticides *viz*., chlorpyriphos, carbofuran and imidacloprid; three herbicides *viz*., pendimethalin, quizolofop ethyl and imazethapyr; eight fertilizers *viz*., urea, ammonium sulphate, calcium ammonium nitrate (CAN), single super phosphate (SSP), diammonium phosphate (DAP), muriate of potash (MOP), zinc sulphate, magnesium sulphate and one amendment gypsum were used to assess their toxicity at field concentrations on *T. harzianum* Th_4 *in vitro* by using poisoned food technique (Nene and Thapliyal, 1993). Radial growth of *T. harzianum* Th_4 was recorded after 24, 48 and 72 h of incubation and per cent inhibition of growth over control on third day was calculated by using the formula given by Vincent (1927).

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

Where,

I = per cent inhibition.

C = growth of Th_4 in unamended medium.

T = growth of Th_4 in amended medium

RESULTS AND DISCUSSION

Effect of agrochemicals on radial growth of Th_4

Fungicides

In fungicide unamended medium, *i.e.,* in control plate, Th_4 radial growth attained a diameter of 2.98 cm after 24 h of incubation. At 48 h of incubation, the radial growth was 6.0 cm and by 72 h of incubation it occupied entire Petri plate, *i.e.,* 9.0 cm radial growth (Table 1). When the medium was amended with test fungicides, significant differences were observed in Th_4 growth.

All the fungicides showed a significant inhibition of Th_4 over check on all the three days of observation (Table 1). Carbendazim did not allow growth initiation after one day of incubation and was significantly the most toxic compared to other fungicides. Growth of Th_4 in mancozeb (0.15 cm) and captan amended medium (0.16 cm) was on a par but was significantly lesser than that in copper oxychloride (0.31 cm), which in turn was significantly lesser than the radial growth in metalaxyl (0.58 cm) amended medium. Growth of Th_4 in carbendazim (0.31 cm) and captan amended medium (0.35 cm) was on a par but significantly lesser than that in metalaxyl (1.00 cm), which in turn significantly lesser than the radial growth in copper oxychloride (1.84 cm) and mancozeb (1.69 cm) amended medium, both copper oxychloride and mancozeb were on a par with each other on second day of incubation. Carbendazim (0.31 cm) amended medium did not show any increase in radial growth and growth of Th_4 remained static after three days of incubation. Growth of Th_4 in carbendazim (0.31 cm) and captan (0.50 cm) amended medium was significantly lesser than copper oxychloride (2.59 cm). Mancozeb (1.71 cm) and metalaxyl (1.70 cm) amended medium was on a par with each other when radial growth on third day was compared. Highest per cent inhibition was observed in carbendazim (96.53) followed by captan (94.44), metalaxyl (81.11), mancozeb (80.97) and copper oxychloride (71.25).

Severe toxic effect of carbendazim (Gupta, 2004; Khan and Saleem, 2007 and Ashwini *et al.,* 2012), captan (Vijayaraghavan and Abraham, 2004; Bagwan, 2010), mancozeb (Ranganath swamy *et al*., 2012; Bagwan, 2010; Madhavi *et al*., 2008 and Sharma and Dureja, 2004) and copper oxychloride (Ashwini *et al*., 2012; Sarkar *et al*., 2010 and Bagwan, 2010) was demonstrated earlier.

Insecticides

All the insecticides except imidacloprid significantly reduced the radial growth of Th_4 on all days of observations (Table 2). Chlorpyriphos was found toxic to Th_4 in comparison to other insecticides as no radial growth was observed one day after incubation. Imidacloprid (2.81 cm) was found to have an initial growth promoting property on Th_4 as the radial growth was found to be significantly more than in chemical unamended (1.88 cm) medium. Growth of Th_4 in carbofuran (1.51 cm) amended medium was significantly lesser than in check. On the second day chlorpyriphos continued to be toxic to Th_4 in comparison to other insecticides with a radial growth of 0.39 cm only while diameter of growth of Th_4 in carbofuran and imidacloprid amended medium and chemical unamended medium was 4.26 cm, 6.09 cm and 6.00 cm, respectively. Radial growth of Th_4 continued to be significantly lesser in chlorpyriphos amended medium (1.01 cm) than in medium with imidacloprid or control in which growth completely occupied the plate (9.00 cm) and on a par with each other. Sparse mycelium of Th_4 was observed in carbofuran (7.98 cm) amended medium on third day of incubation. Imidacloprid amended medium showed no inhibition on three days after incubation. Chlorpyriphos (88.75) recorded the highest per cent inhibition followed by carbofuran (11.39).

Highly toxic effect of chlorpyriphos (Vijayaraghavan and Abraham, 2004; Gupta, 2004, and Sharma and Dureja, 2004), lesser toxicity of carbofuran (Ranganathswamy *et al*., 2012) and least toxicity of imidacloprid (Ranganathswamy *et al*., 2012 and Madhavi *et al*., 2008) was demonstrated earlier.

Table 1. Effect of fungicides on the radial growth of *T. harzianum* Th_4 isolate.

S. No.	Fungicides	Conc. (%)	Radial growth (cm)			Inhibition over control (%) as on 3rd day
			Day1	Day2	Day3	
1.	Copper oxychloride	0.3	0.31 (1.15)^c	1.84 (1.68)^c	2.59 (1.89)^c	71.25
2.	Mancozeb	0.25	0.15 (1.07)^b	1.69 (1.64)^c	1.71 (1.65)^b	80.97
3.	Carbendazim	0.2	0.00 (1.00)^a	0.31 (1.15)^a	0.31 (1.15)^a	96.53
4.	Metalaxyl	0.2	0.58 (1.25)^d	1.00 (1.41)^b	1.70 (1.64)^b	81.11
5.	Captan	0.2	0.16 (1.08)^b	0.35 (1.16)^a	0.50 (1.22)^a	94.44
6.	Check		2.98 (1.99)^e	6.00 (2.65)^d	9.00 (3.16)^d	-
	SEm±		0.01	0.03	0.02	
	CD (P d" 0.01)		0.02	0.08	0.07	
	CV (%)		1.96	3.32	2.55	

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

Table 2. Effect of insecticides on the radial growth of *T. harzianum* Th_4 isolate.

Insecticides	Conc. (%)	Radial growth (cm)			Inhibition over control (%) as on 3rd day
		Day1	Day2	Day3	
Chlorpyriphos	0.25	0.00 (1.00)^a	0.39 (1.18)^a	1.01 (1.42)^a	88.75
Carbofuran	0.25	1.51 (1.59)^b	4.26 (2.29)^b	7.98 (3.00)^b	11.39
Imidacloprid	0.025	2.81 (1.95)^d	6.09 (2.66)^c	9.00 (3.16)^c	0.00
Check		1.88 (1.70)^c	6.00 (2.65)^c	9.00 (3.16)^c	-
SEm±		0.01	0.02	0.01	
CD (P d" 0.01)		0.04	0.07	0.04	
CV (%)		1.74	2.18	0.91	

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

Herbicides

All the herbicides except imazethapyr significantly reduced the radial growth of Th_4 on all days of observations (Table 3). Pendimethalin was found highly toxic to Th_4 in comparison to other herbicides. No radial growth of Th_4 was observed even after three days of incubation on pendimethalin amended medium. Quizolofop ethyl was also found toxic to Th_4 only initially as no radial growth was observed one day after incubation whereas imazethapyr was found to have an initial growth promoting property on Th_4 as the radial growth (2.14 cm) was found to be significantly more than in chemical unamended (0.65 cm) medium. On the second day, radial growth of Th_4 continued to be significantly lesser in quizaolofop ethyl (0.23 cm) amended medium in comparison to chemical unamended (6.06 cm) medium, whereas imazethapyr (6.49 cm) was found to have significantly higher radial growth. On the third day, Th_4 growth on imazethapyr (9.00 cm) and chemical unamended (9.00 cm) medium was on a par with each other while on quizolofop ethyl medium growth (0.29 cm) was significantly and substantially lesser. When mean percent inhibition over control was compared, pendimethalin amended medium showed 100 per cent inhibition followed by quizolofop ethyl (96.81%) and no inhibition in growth of Th_4 was found in imazethapyr amended medium after three days of incubation of Th_4.

Higher toxicity of pendimethalin to *Trichoderma* isolates reported by Mahalaxmi *et al.,* (2008) and Ranganathswamy *et al*., (2012).

Fertilizers

All the fertilizers except MOP, SSP and gypsum significantly reduced the radial growth of Th_4 on first day of incubation (Table 4). Zinc sulphate was found more toxic to Th_4 than other fertilizers as no radial growth was observed one day after incubation followed by DAP (0.23 cm) amended medium. Growth on urea (1.23 cm), ammonium sulphate (1.83 cm), CAN (1.15 cm), magnesium sulphate (1.60 cm) amended medium and chemical unamended (1.88 cm) medium were on a par with each other. SSP (5.13 cm), MOP (2.65 cm) and gypsum (5.03 cm) amended medium was found to have an initial growth promoting property on Th_4 as the radial growth was found to be significantly more than in chemical unamended (1.88 cm) medium. On the second day, all the fertilizers except SSP, gypsum and MOP significantly reduced the radial growth of Th_4. Zinc sulphate (0.33 cm) was found toxic to Th_4 in comparison to other fertilizers. SSP (9.00 cm) and gypsum (9.00 cm) amended medium was found to have continuous growth promoting property on Th_4 as the radial growth was found to be more than in chemical unamended medium (5.99 cm) medium. Growth of Th_4 in MOP (5.83 cm) and chemical unamended was on a par. On the third day, all the fertilizers except SSP, MOP, magnesium sulphate and gypsum significantly reduced the radial growth of Th_4. Zinc sulphate (0.41 cm) was found toxic to Th_4 in comparison to other fertilizers. SSP (9.00 cm), MOP (9.00 cm), magnesium sulphate (9.00 cm), gypsum (9.00 cm) and chemical unamended (9.00 cm) medium was on a par with each other and occupied the entire Petri plate. Urea (3.54 cm), ammonium sulphate (7.74 cm), CAN (6.24 cm) and DAP (4.31 cm) were found toxic to Th_4 and significantly reduced growth of Th_4 compared to check. When mean per cent inhibition over control was compared, zinc sulphate (95.42%) found toxic to Th_4 followed by urea (60.69%), DAP (52.08%), CAN (30.69%) and ammonium sulphate (14.03%) and no inhibitory effect was observed in SSP, MOP, magnesium sulphate and gypsum after three days of incubation.

Higher toxicity of zinc sulphate may be attributed to heavy metal toxicity leading to inhibition of several essential enzymes required for growth and survival of the test antagonists. Low inhibitory effect of ammonium sulphate on Th_4 isolate may be due to the availability of ammonium form of nitrogen which is most favourable for mycelial growth. Gypsum and SSP amended medium showed profuse growth in comparison to check plate. Compatibility of gypsum with Th_4 can be attributed to reduction of pH of medium by gypsum thereby creating favourable conditions for the growth of Th_4.

Toxic effect of zinc sulphate (Sundaravadana and Alice, 2006), urea and DAP (Ranganathswamy *et al*., 2012), compatibility of CAN (Johnson *et al.,* 2008), non-inhibitory effect of ammonium sulphate (Vijayaraghavan and Abraham, 2004), compatibility of gypsum, SSP and

Table 3. Effect of herbicides on the radial growth of *T. harzianum* Th_4 isolate.

Herbicides	Conc. (%)	Radial growth (cm)			Inhibition over control (%) as on 3rd day
		Day1	Day2	Day3	
Pendimethalin	0.66	0.00 (1.00)[a]	0.00 (1.00)[a]	0.00 (1.00)[a]	100.00
Quizolofop ethyl	0.2	0.00 (1.00)[a]	0.23 (1.93)[b]	0.29 (2.02)[b]	96.81 0.00 -
Imazethapyr	0.1	2.14 (3.07)[c]	6.49 (3.97)[d]	9.00 (4.30)[c]	
check		0.65 (2.37)[b]	6.06 (3.90)[c]	9.00 (4.30)[c]	
SEm±		0.01	0.01	0.02	
CD (P d"0.01)		0.03	0.04	0.05	
CV (%)		1.11	0.97	1.07	

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

Table 4: Effect of fertilizers on the radial growth of *T. harzianum* Th_4 isolate.

S. No.	Fertilizers	Conc. (%)	Radial growth (cm)			Inhibition over control (%) as on 3rd day
			Day1	Day2	Day3	
1.	Urea	2	1.23 (1.49)[b]	2.71 (1.93)[c]	3.54 (2.13)[b]	60.69
2.	Ammonium sulphate	2	1.83 (1.68)[b]	4.88 (2.42)[f]	7.74 (2.96)[e]	14.03
3.	Calcium ammonium nitrate	2	1.15 (1.47)[b]	3.48 (2.12)[d]	6.24 (2.69)[d]	30.69
4.	Single super phosphate	2	5.13 (2.47)[d]	9.00 (3.16)[h]	9.00 (3.16)[f]	0.00
5.	Diammonium phosphate	2	0.23 (1.11)[a]	1.18 (1.47)[b]	4.31 (2.30)[c]	52.08
6.	Muriate of potash	2	2.65 (1.91)[c]	5.83 (2.61)[g]	9.00 (3.16)[f]	0.00
7.	Zinc sulphate	0.2	0.00 (1.00)[a]	0.33 (1.15)[a]	0.41 (1.19)[a]	95.42
8.	Magnesium sulphate	0.2	1.60 (1.61)[b]	4.46 (2.34)[e]	9.00 (3.16)[f]	0.00
9.	Gypsum	2	5.03 (2.45)[d]	9.00 (3.16)[h]	9.00 (3.16)[f]	0.00
10.	Check		1.88 (1.70)[b]	5.99 (2.64)[g]	9.00 (3.16)[f]	-
	SEm±		0.04	0.02	0.01	
	CD (P d" 0.01)		0.10	0.05	0.04	
	CV (%)		4.16	1.48	1.10	

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

MOP (Bhai and Thomas, 2010; Ranganathswamy *et al*., 2012 and Vijayaraghavan and Abraham, 2004) was demonstrated earlier.

It can be elucidated from the results that the less or insensitive isolates of *Trichoderma* can be integrated with agrochemicals *viz*., imidacloprid, imazethapyr, SSP, MOP, magnesium sulphate and gypsum for the management of soil borne plant diseases. However, the toxicity of these chemicals to the biocontrol agent in soil system needs to be ascertained before they are recommended for field adoption.

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(Received on 29.10.2013 and revised on 04.06.2014)