

**Research Article**

**EFFECT OF AGROCHEMICALS ON *TRICHODERMA HARZIANUM* (Th<sub>4</sub> ISOLATE) AND ITS BIOCONTROL POTENTIAL AGAINST CHICKPEA COLLAR ROT CAUSED BY *SCLEROTIUM ROLFSII***

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**ABSTRACT**

Pot culture experiments were conducted to study the effect of most commonly used selected agrochemicals viz., carbendazim (0.2%), chlorpyrifos (0.25%), pendimethalin (0.66%) and zinc sulphate (0.2%) alone and in combination with amendment gypsum (2%) on the biocontrol efficacy of *Trichoderma harzianum* (Th<sub>4</sub>) as seed treatment/ soil application against collar rot of chickpea caused by *Sclerotium rolfsii*. Among all seed treatments, seed treatment with Th<sub>4</sub>- chlorpyrifos- *S. rolfsii* had shown the highest per cent germination (96.75), high plant stand per cent (55.93) and high colony forming units (cfu) recovery ( $20.67 \times 10^6$  cfu/g soil) and from among all soil application treatments, soil application with Th<sub>4</sub>-chlorpyrifos- *S. rolfsii* recorded the high per cent germination (83.07) and soil application with Th<sub>4</sub>-chlorpyrifos- gypsum- *S. rolfsii* had shown high plant stand per cent (25.42) and high cfu recovery of  $20.67 \times 10^6$  cfu/g soil.

**Keywords:** Agrochemicals, *Trichoderma Harzianum*, *Sclerotium Rolfsii*

**INTRODUCTION**

Soil borne plant pathogenic fungi such as *Pythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia*, *Sclerotium* and *Fusarium* are widely distributed in soil and affect various crops of economic importance (Chet *et al.*, 1997).

*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). Biological control using *Trichoderma* offers a novel approach when applied alone or in combination with other management practices (Papavizas, 1985).

Species of *Trichoderma* are being used either through seed treatment or soil application to manage several soil borne plant diseases.

Hence, the present investigation was conducted to study the compatibility of selected agrochemicals alone or in combination with gypsum on the biocontrol efficacy of *T. harzianum* (Th<sub>4</sub>) as seed treatment / soil application against collar rot of chickpea caused by *S. rolfsii*.

**MATERIALS AND METHODS**

*Trichoderma harzianum* (Th<sub>4</sub>) obtained from cotton ecosystem, which was maintained in Department of plant pathology, Agricultural College, Bapatla was used in the present investigation.

Most commonly used agrochemicals viz., carbendazim (0.2%), chlorpyrifos (0.25%), pendimethalin (0.66%) and zinc sulphate (0.2%) and one amendment gypsum (2%) were used to study the compatibility and biocontrol efficacy of *T. harzianum* (Th<sub>4</sub>) seed treatment and soil application against *S. rolfsii* causing chickpea collar rot. Seed treatment of Th<sub>4</sub> was done at  $10^8$  spores/ml concentrations, while Th<sub>4</sub> soil application was done at 10 g/pot and *S. rolfsii* at six g/pot, which were mass multiplied on sorghum grains of 15 days old culture was used. Twenty chickpea seeds were sown in the earthen pots under green house conditions.

Observations were recorded on the number of seeds germinated and plant stand. Rhizosphere soil samples were collected at 30 DAS to estimate *T. harzianum* Th<sub>4</sub> population using *Trichoderma* selective medium (TSM). The following formulae were used to assess treatment effects in pot culture.

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$$\text{Plant stand (\%)} = \frac{\text{Number of plants in treatments}}{\text{Plant stand in absolute check}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Number of plants in absolute check} - \text{Number of plants in treatments}}{\text{Number of plants in absolute check}} \times 100$$

$$\text{Disease Control (\%)} = \frac{\text{Mortality \% in pathogen check} - \text{Mortality \% in treatment}}{\text{Mortality \% in pathogen check}} \times 100$$

## RESULTS AND DISCUSSION

### *Trichoderma Harzianum TH<sub>4</sub> as Seed Treatment against S. Rolfsii in Chickpea in Pot Experiment*

In the absence of gypsum, when comparisons were made with absolute check (pathogen uninoculated) (19.67 plants/pot) at 30 DAS,

all the treatments were significantly different from absolute check (pathogen uninoculated).

Among the treatments, seed treatment of Th<sub>4</sub> - chlorpyrifos - *S. rolfsii* had shown significantly higher plant stand (11 plants/pot),

which may be due to less inhibitory effect of chlorpyrifos on population of Th<sub>4</sub>, so that Th<sub>4</sub> could control *S. rolfsii* and decrease the per cent seedling mortality,

whereas seed treatment of Th<sub>4</sub> - zinc sulphate - *S. rolfsii* had lower plant stand (4 plants/pot) showing high incompatibility with Th<sub>4</sub> seed treatment (Table 1).

Germination per cent of seed treatment with Th<sub>4</sub> - chlorpyrifos - *S. rolfsii* was on a par with absolute check (100.00) remaining treatments were significantly less compared to absolute check.

*S. rolfsii* inoculum had a negative effect.

However, the negative effect can be nullified by addition of chlorpyrifos, which may be ascribed to rapid degradation of chlorpyrifos when applied to soil without ill effect on Th<sub>4</sub> population.

This was in agreement with Jebakumar *et al.*, (2000), Singh *et al.*, (2010) and Vimala *et al.*, (2010).

Seed treatment of Th<sub>4</sub> - chlorpyrifos - *S. rolfsii* (23.00 × 10<sup>6</sup> cfu/g soil) has recorded significantly higher mean Th<sub>4</sub> population at 30 DAS.

This can be due to better rhizosphere colonization of Th<sub>4</sub> and its bioefficacy against *S. rolfsii* (Table 1).

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**Table 1: Effect of selected agrochemicals alone and in combination with amendment on the biocontrol efficacy of *T. harzianum* Th<sub>4</sub> as seed treatment against *S. rolfii* in chickpea**

S. No.	Treatments	Germination %	Plant stand at 30 DAS	Plant stand (%)	Mortality (%)	Disease control (%)	Th <sub>4</sub> population (×10 <sup>6</sup> cfu/g soil) at 30 DAS
1	ST - Carbendazim - <i>S. rolfii</i>	73.33 (58.89)	7.33 (2.89)	37.29	62.71	31.48	17.00 (4.24)
2	ST - Chlorpyrifos - <i>S. rolfii</i>	96.57 (79.59)	11.00 (3.46)	55.93	44.07	51.85	23.00 (4.90)
3	ST - Pendimethalin - <i>S. rolfii</i>	59.21 (50.29)	6.00 (2.65)	30.51	69.49	24.07	13.00 (3.74)
4	ST - Zinc sulphate - <i>S. rolfii</i>	62.37 (52.14)	4.00 (2.24)	20.34	79.66	12.96	10.00 (3.32)
5	ST - Carbendazim - Gypsum - <i>S. rolfii</i>	69.74 (56.60)	2.00 (1.73)	10.17	89.83	1.85	13.33 (3.79)
6	ST - Chlorpyrifos - Gypsum - <i>S. rolfii</i>	84.65 (66.91)	8.00 (3.00)	40.68	59.32	35.19	13.00 (3.74)
7	ST - Pendimethalin - Gypsum - <i>S. rolfii</i>	71.32 (57.59)	1.33 (1.53)	6.78	93.22	-1.85	0.00 (1.00)
8	ST - Zinc sulphate - Gypsum - <i>S. rolfii</i>	38.39 (38.60)	0.00 (1.00)	0.00	100.00	-9.26	0.00 (1.00)
9	ST - <i>S. rolfii</i> - Gypsum	81.75 (64.69)	1.00 (1.41)	5.08	94.92	-3.70	0.00(1.00)
10	ST - <i>S. rolfii</i>	52.72 (46.54)	0.00 (1.00)	0.00	100.00	-9.26	7.00 (2.83)
11	<i>S. rolfii</i> - gypsum	47.28 (43.42)	2.00 (1.73)	10.17	89.83	1.85	0.00 (1.00)
12	Pathogen check	79.82 (63.28)	1.67 (1.63)	8.47	91.53		0.33 (1.15)
13	Absolute check (Pathogen uninoculated)	100.00 (89.96)	19.67 (4.58)	-	-	-	0.33 (1.15)
CD (P ≤ 0.01)		13.50	0.48				0.24
CV (%)		11.36	13.60				5.70

\*Each treatment replicated thrice

ST - seed treatment of Th<sub>4</sub> @ 10<sup>8</sup> spores/ml

20 seeds were sown per pot

Figures in parentheses are square root transformed value

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**Table 2: Effect of selected agrochemicals alone and in combination with amendment on the biocontrol efficacy of *T. harzianum* Th<sub>4</sub> as soil application against *S. rolfsii* in chickpea**

S. No.	Treatments	Germination %	Plant stand at 30 DAS	Plant stand (%)	Mortality (%)	Disease control (%)	Th <sub>4</sub> population (×10 <sup>6</sup> cfu/g soil) at 30 DAS
1	SA - Carbendazim - <i>S. rolfsii</i>	47.46 (43.52)	0.67 (1.29)	3.39	96.61	-5.56	3.33 (2.08)
2	SA - Chlorpyrifos - <i>S. rolfsii</i>	83.07 (65.68)	1.00 (1.41)	5.08	94.92	-3.70	16.33 (4.16)
3	SA - Pendimethalin - <i>S. rolfsii</i>	57.63 (49.37)	1.33 (1.53)	6.78	93.22	-1.85	8.00 (3.00)
4	SA - Zinc sulphate - <i>S. rolfsii</i>	45.88 (42.62)	0.00 (1.00)	0.00	100.00	-9.26	7.33 (2.89)
5	SA - Carbendazim - Gypsum - <i>S. rolfsii</i>	57.46 (49.27)	1.33 (1.53)	6.78	93.22	-1.85	1.33 (1.53)
6	SA - Chlorpyrifos - Gypsum - <i>S. rolfsii</i>	67.81 (55.41)	5.00 (2.45)	25.42	74.58	18.52	20.67 (4.65)
7	SA - Pendimethalin - Gypsum - <i>S. rolfsii</i>	52.28 (46.29)	3.33 (2.08)	16.95	83.05	9.26	15.00 (4.00)
8	SA - Zinc sulphate - Gypsum - <i>S. rolfsii</i>	56.05 (48.46)	0.00 (1.00)	0.00	100.00	-9.26	0.00 (1.00)
9	SA - <i>S. rolfsii</i> - Gypsum	42.37 (40.59)	2.33 (1.83)	11.86	88.14	3.70	3.67 (2.16)
10	SA - <i>S. rolfsii</i>	45.79 (42.57)	3.67 (2.16)	18.64	81.36	11.11	12.00 (3.61)
11	<i>S. rolfsii</i> - gypsum	47.28 (43.32)	2.00 (1.73)	10.17	89.83	1.85	4.33 (2.31)
12	Pathogen check	79.82 (63.28)	1.67 (1.63)	8.47	91.53	-	1.00 (1.41)
13	Absolute check (Pathogen uninoculated)	100.00 (89.96)	19.67 (4.55)	-	-	-	0.33 (1.15)
CD (P ≤ 0.01)		5.31	0.24				0.37
CV (%)		6.05	7.69				8.52

\*Each treatment replicated thrice

SA- soil application of Th<sub>4</sub> @ 6g/6Kg soil pot

20 seeds were sown per pot

Figures in parentheses are square root transformed value

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Among gypsum amended treatments, Th<sub>4</sub> seed treatment – gypsum – chlorpyrifos - *S. rolfsii* showed significantly higher plant stand (8 plants/pot) at 30 DAS where as complete seedling mortality was observed in Th<sub>4</sub> seed treatment - zinc sulphate - gypsum - *S. rolfsii* (0 plants/pot) showed that zinc sulphate had severe toxic effect on Th<sub>4</sub> (Table 1). This was in agreement with Gurjar *et al.*, (2003). Th<sub>4</sub> seed treatment - pendimethalin - gypsum - *S. rolfsii*, Th<sub>4</sub> seed treatment- zinc sulphate - gypsum - *S. rolfsii*, Th<sub>4</sub> seed treatment - *S. rolfsii* - gypsum and *S. rolfsii* – gypsum there was no recovery of Th<sub>4</sub> from soil. It may be due to toxicity of pendimethalin and zinc sulphate to the population of Th<sub>4</sub> (Table 1).

Among all the treatments, the highest plant stand (55.93%) and highest disease control (51.85%) observed in Th<sub>4</sub> seed treatment- chlorpyrifos - *S. rolfsii*. Highest mortality (100%) and lowest plant stand (0%) was observed in Th<sub>4</sub> seed treatment - zinc sulphate - gypsum - *S. rolfsii* (Table 1).

### **Trichoderma Harzianum Th<sub>4</sub> as Soil Application against *s. rolfsii* in Chickpea in Pot Experiment**

Among all the gypsum unamended treatments Th<sub>4</sub> soil application - *S. rolfsii* had significantly higher plant stand (3.67 plants/pot) at 30 DAS compared to all other treatments. In all the treatments plant stand was significantly lower when comparisons were made with absolute check (19.67 plants/pot) (Table 2).

In gypsum amended treatments, plant stand at 30 DAS was significantly lower when comparisons were made with absolute check (19.67 plants/pot). Th<sub>4</sub> soil application - chlorpyrifos - gypsum - *S. rolfsii* had higher population (5.00 plants/pot). When both Th<sub>4</sub> and gypsum were directly applied to the soil, Th<sub>4</sub> had more chances to thicken its cell wall, so that the toxic chemical permeability into it is blocked there by facilitating the proliferation of Th<sub>4</sub> in the soil as reported by Jebakumar *et al.*, (2000); Singh *et al.*, (2010) and Vimala *et al.*, (2010) (Table 2). Among all soil application treatments, the high germination per cent was observed in soil application with Th<sub>4</sub>- chlorpyrifos- *S. rolfsii* (83.07) but all were significantly less when compared to absolute check (100.00)

In both seed treatment and soil application, zinc sulphate amended treatments were more toxic to Th<sub>4</sub> by facilitating favorable environment for rapid multiplication of the *S. rolfsii* leading to complete seedling mortality at 30 DAS. It can be inferred that zinc sulphate was found more toxic to Th<sub>4</sub>, so it is not advisable to use zinc sulphate in combination with Th<sub>4</sub> with or without gypsum as it is proved to be toxic to Th<sub>4</sub> (Table 1 and 2).

Highest plant stand was found in Th<sub>4</sub> soil application- chlorpyrifos - gypsum - *S. rolfsii* (25.42%) when comparisons were made among all treatments with absolute check (100%). Chlorpyrifos did not showed any toxic effect on Th<sub>4</sub> and gypsum facilitated Th<sub>4</sub> proliferation in the soil. Lowest mortality was observed in Th<sub>4</sub> soil application - chlorpyrifos - gypsum - *S. rolfsii* (74.58%) when comparisons were made among all treatments with pathogen check (91.53%) with a disease control per cent of 18.52. Complete mortality was observed in zinc sulphate amended treatments when compared to pathogen check (91.53%) which proved that zinc sulphate was more toxic to Th<sub>4</sub>. (Table 2).

When Th<sub>4</sub> population was estimated and means comparisons of cfu at 30 DAS were made, significant difference was observed among all the treatments. Higher Th<sub>4</sub> population was observed in Th<sub>4</sub> soil application - chlorpyrifos - gypsum - *S. rolfsii* ( $20.67 \times 10^6$  cfu/g soil) followed by Th<sub>4</sub> soil application - chlorpyrifos - *S. rolfsii* ( $16.33 \times 10^6$  cfu/g soil) and Th<sub>4</sub> soil application - pendimethalin - gypsum - *S. rolfsii* ( $15.00 \times 10^6$  cfu/g soil) (Table 2). Thus, the present investigation has revealed that, agrochemicals such as carbendazim, chlorpyrifos, pendimethalin and zinc sulphate were toxic to Th<sub>4</sub> and were ineffective in control of collar rot pathogen *S. rolfsii*. In chlorpyrifos applied treatments the plant stand per cent and germination per cent were high both in seed treatment and soil application of Th<sub>4</sub> due to the subsequent protective effect of Th<sub>4</sub> which must have increased with the decreasing effect of chlorpyrifos over time, but remaining three agrochemicals proved toxic to Th<sub>4</sub>, among all the selected agrochemicals zinc sulphate proved to be more toxic to Th<sub>4</sub>.

## REFERENCES

**Chet I, Inar J and Hardar I (1997).** Fungal antagonists and mycoparasites. In: *The Mycota iv: Environmental and Microbial Relationships*, edited by Wicklow DT and Soderstorm B (Springer-Verlag) Berlin 165-184.

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

**Gurjar RBS, Bansal RK and Gupta RBL (2003).** Effect of soil amendments on collar rot of chilli caused by *Sclerotium rolfsii* Sacc. *Journal of Mycology and Plant Pathology* **33**(3) 482.

**Jebakumar RS, Anandaraj M and Sharma YR (2000).** Compatibility of phorate and chlorpyrifos with *Trichoderma harzianum* applied for integrated disease management in black pepper (*Piper nigrum* L.). *Journal of Spices and Aromatic Crops* **9**(2) 111-115.

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

**Singh N, Srivastava A, Srivastava PC and Srivastava N (2010).** Role of soil amendments in dissipation of chlorpyrifos in mollisols. *Pesticide Research Journal* **22**(2) 139-142.

**Vimala R, Selven SC, Murugesan N and Ramalingam A (2009).** Development of a management strategy against stem weevil and root rot complex in cotton. *Journal of Biopesticides* **2**(1) 18-21.