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Research Article

EFFECT OF AGROCHEMICALS ON *TRICHODERMA HARZIANUM* (Th_4 ISOLATE) AND ITS BIOCONTROL POTENTIAL AGAINST CHICKPEA COLLAR ROT CAUSED BY *SCLEROTIUM ROLFSII*

***Neeraja B., Manoj Kumar V., Krishna Prasadji J. and Anil Kumar P.**
Department of Plant Pathology, Agricultural College, Bapatla, Andhra Pradesh 522 101
**Author for Correspondence*

ABSTRACT
Pot culture experiments were conducted to study the effect of most commonly used selected agrochemicals *viz*., carbendazim (0.2%), chlorpyriphos (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_4) as seed treatment/ soil application against collar rot of chickpea caused by *Sclerotium rolfsii*. Among all seed treatments, seed treatment with Th_4- chlorpyriphos- *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 (20.67×10^6 cfu/g soil) and from among all soil application treatments, soil application with Th_4-chlorpyriphos- *S. rolfsii* recorded the high per cent germination (83.07) and soil application with Th_4-chlorpyriphos- gypsum- *S. rolfsii* had shown high plant stand per cent (25.42) and high cfu recovery of 20.67×10^6 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_4) as seed treatment / soil application against collar rot of chickpea caused by *S. rolfsii*.

MATERIALS AND METHODS
Trichoderma harzianum (Th_4) 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%), chlorpyriphos (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_4) seed treatment and soil application against *S. rolfsii* causing chickpea collar rot. Seed treatment of Th_4 was done at 10^8 spores/ml concentrations, while Th_4 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_4 population using Trichoderma selective medium (TSM). The following formulae were used to assess treatment effects in pot culture.

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

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

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

RESULTS AND DISCUSSION

Trichoderma Harzianum TH_4 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_4 - chlorpyriphos - *S. rolfsii* had shown significantly higher plant stand (11 plants/pot),

which may be due to less inhibitory effect of chlorpyriphos on population of Th_4, so that Th_4 could control *S. rolfsii* and decrease the per cent seedling mortality,

whereas seed treatment of Th_4 - zinc sulphate - *S. rolfsii* had lower plant stand (4 plants/pot) showing high incompatibility with Th_4 seed treatment (Table 1).

Germination per cent of seed treatment with Th_4 - chlorpyriphos - *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 chlorpyriphos, which may be ascribed to rapid degradation of chlorpyriphos when applied to soil without ill effect on Th_4 population.

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

Seed treatment of Th_4 - chlorpyriphos - *S. rolfsii* (23.00×10^6 cfu/g soil) has recorded significantly higher mean Th_4 population at 30 DAS.

This can be due to better rhizosphere colonization of Th_4 and its bioefficacy against *S. rolfsii* (Table 1).

Table 1: Effect of selected agrochemicals alone and in combination with amendment on the biocontrol efficacy of *T. harzianum* Th_4 as seed treatment against *S. rolfsii* in chickpea

S. No.	Treatments	Germination %	Plant stand at 30 DAS	Plant stand (%)	Mortality (%)	Disease control (%)	Th_4 population ($\times 10^6$ cfu/g soil) at 30 DAS
1	**ST - Carbendazim - *S. rolfsii***	73.33 (58.89)	7.33 (2.89)	37.29	62.71	31.48	17.00 (4.24)
2	**ST - Chlorpyriphos - *S. rolfsii***	96.57 (79.59)	11.00 (3.46)	55.93	44.07	51.85	23.00 (4.90)
3	**ST - Pendimethalin - *S. rolfsii***	59.21 (50.29)	6.00 (2.65)	30.51	69.49	24.07	13.00 (3.74)
4	**ST - Zinc sulphate - *S. rolfsii***	62.37 (52.14)	4.00 (2.24)	20.34	79.66	12.96	10.00 (3.32)
5	**ST - Carbendazim - Gypsum - *S. rolfsii***	69.74 (56.60)	2.00 (1.73)	10.17	89.83	1.85	13.33 (3.79)
6	**ST - Chlorpyriphos - Gypsum - *S. rolfsii***	84.65 (66.91)	8.00 (3.00)	40.68	59.32	35.19	13.00 (3.74)
7	**ST - Pendimethalin - Gypsum - *S. rolfsii***	71.32 (57.59)	1.33 (1.53)	6.78	93.22	-1.85	0.00 (1.00)
8	**ST - Zinc sulphate - Gypsum - *S. rolfsii***	38.39 (38.60)	0.00 (1.00)	0.00	100.00	-9.26	0.00 (1.00)
9	**ST - *S. rolfsii* - Gypsum**	81.75 (64.69)	1.00 (1.41)	5.08	94.92	-3.70	0.00(1.00)
10	**ST - *S. rolfsii***	52.72 (46.54)	0.00 (1.00)	0.00	100.00	-9.26	7.00 (2.83)
11	***S. rolfsii* - 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 ($P \leq 0.01$)		13.50	0.48				0.24
CV (%)		11.36	13.60				5.70

**Each treatment replicated thrice*
ST - seed treatment of Th_4 @ 10^8 spores/ml
20 seeds were sown per pot
Figures in parentheses are square root transformed value

Table 2: Effect of selected agrochemicals alone and in combination with amendment on the biocontrol efficacy of *T. harzianum* Th_4 as soil application against *S. rolfsii* in chickpea

S. No.	Treatments	Germination %	Plant stand at 30 DAS	Plant stand (%)	Mortality (%)	Disease control (%)	Th_4 population ($\times 10^6$ cfu/g soil) at 30 DAS
1	**SA - Carbendazim - *S. rolfsii***	47.46 (43.52)	0.67 (1.29)	3.39	96.61	-5.56	3.33 (2.08)
2	**SA - Chlorpyriphos - *S. rolfsii***	83.07 (65.68)	1.00 (1.41)	5.08	94.92	-3.70	16.33 (4.16)
3	**SA - Pendimethalin - *S. rolfsii***	57.63 (49.37)	1.33 (1.53)	6.78	93.22	-1.85	8.00 (3.00)
4	**SA - Zinc sulphate - *S. rolfsii***	45.88 (42.62)	0.00 (1.00)	0.00	100.00	-9.26	7.33 (2.89)
5	**SA - Carbendazim - Gypsum - *S. rolfsii***	57.46 (49.27)	1.33 (1.53)	6.78	93.22	-1.85	1.33 (1.53)
6	**SA - Chlorpyriphos - Gypsum - *S. rolfsii***	67.81 (55.41)	5.00 (2.45)	25.42	74.58	18.52	20.67 (4.65)
7	**SA - Pendimethalin - Gypsum - *S. rolfsii***	52.28 (46.29)	3.33 (2.08)	16.95	83.05	9.26	15.00 (4.00)
8	**SA - Zinc sulphate - Gypsum - *S. rolfsii***	56.05 (48.46)	0.00 (1.00)	0.00	100.00	-9.26	0.00 (1.00)
9	**SA - *S. rolfsii* - Gypsum**	42.37 (40.59)	2.33 (1.83)	11.86	88.14	3.70	3.67 (2.16)
10	**SA - *S. rolfsii***	45.79 (42.57)	3.67 (2.16)	18.64	81.36	11.11	12.00 (3.61)
11	***S. rolfsii* - 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 ($P \leq 0.01$)		5.31	0.24				0.37
CV (%)		6.05	7.69				8.52

**Each treatment replicated thrice*
SA- soil application of Th_4 @ 6g/6Kg soil pot
20 seeds were sown per pot
Figures in parentheses are square root transformed value

Among gypsum amended treatments, Th_4 seed treatment – gypsum – chlorpyriphos - *S. rolfsii* showed significantly higher plant stand (8 plants/pot) at 30 DAS where as complete seedling mortality was observed in Th_4 seed treatment - zinc sulphate - gypsum - *S. rolfsii* (0 plants/pot) showed that zinc sulphate had severe toxic effect on Th_4 (Table 1). This was in agreement with Gurjar *et al.,* (2003). Th_4 seed treatment - pendimethalin - gypsum - *S. rolfsii,* Th_4 seed treatment- zinc sulphate - gypsum - *S. rolfsii,* Th_4 seed treatment - *S. rolfsii* - gypsum and *S. rolfsii* – gypsum there was no recovery of Th_4 from soil. It may be due to toxicity of pendimethalin and zinc sulphate to the population of Th_4 (Table 1).

Among all the treatments, the highest plant stand (55.93%) and highest disease control (51.85%) observed in Th_4 seed treatment- chlorpyriphos - *S. rolfsii*. Highest mortality (100%) and lowest plant stand (0%) was observed in Th_4 seed treatment - zinc sulphate - gypsum - *S. rolfsii* (Table 1).

Trichoderma Harzianum Th₄ as Soil Application against s. rolfsii in Chickpea in Pot Experiment

Among all the gypsum unamended treatments Th_4 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_4 soil application - chlorpyriphos - gypsum - *S. rolfsii* had higher population (5.00 plants/pot). When both Th_4 and gypsum were directly applied to the soil, Th_4 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_4 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_4- chlorpyriphos- *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_4 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_4, so it is not advisable to use zinc sulphate in combination with Th_4 with or without gypsum as it is proved to be toxic to Th_4 (Table 1 and 2).

Highest plant stand was found in Th_4 soil application- chlorpyriphos - gypsum - *S. rolfsii* (25.42%) when comparisons were made among all treatments with absolute check (100%). Chlorpyriphos did not showed any toxic effect on Th_4 and gypsum facilitated Th_4 proliferation in the soil. Lowest mortality was observed in Th_4 soil application - chlorpyriphos - 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_4. (Table 2).

When Th_4 population was estimated and means comparisons of cfu at 30 DAS were made, significant difference was observed among all the treatments. Higher Th_4 population was observed in Th_4 soil application - chlorpyriphos - gypsum - *S. rolfsii* (20.67×10^6 cfu/g soil) followed by Th_4 soil application - chlorpyriphos - *S. rolfsii* (16.33×10^6 cfu/g soil) and Th_4 soil application - pendimethalin - gypsum - *S. rolfsii* (15.00×10^6 cfu/g soil) (Table 2). Thus, the present investigation has revealed that, agrochemicals such as carbendazim, chlorpyriphos, pendimethalin and zinc sulphate were toxic to Th_4 and were ineffective in control of collar rot pathogen *S. rolfsii*. In chlorpyriphos applied treatments the plant stand per cent and germination per cent were high both in seed treatment and soil application of Th_4 due to the subsequent protective effect of Th_4 which must have increased with the decreasing effect of chlorpyriphos over time, but remaining three agrochemicals proved toxic to Th_4, among all the selected agrochemicals zinc sulphate proved to be more toxic to Th_4.

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