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Evaluation of *Trichoderma* spp. Against *Sclerotium rolfsii* Sacc., Causing Stem Rot in Groundnut Crop

Y. Chandra Sekhar[1*], S. Khayum Ahammed[2], T.N.V.K.V.Prasad[3] and R. Sarada Jayalakshmi Devi[4]

[1]Department of Plant Pathology, S.V. Agricultural College, Acharya N G Ranga Agricultural University, Tirupati – 517 502

[2]Regional Agricultural Research Station, Acharya N G Ranga Agricultural University Nandyal – 518 502

[3]Nanotechnology laboratory, Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupati – 517 502

[4]University Librarian, Acharya N G Ranga Agricultural University, Lam, Guntur - 522034

*Corresponding Author E-mail: chandu.008y@gmail.com

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ABSTRACT

Ten Trichoderma spp. isolates were screened for their antagonistic potential against Sclerotim rolfsii (S.r-9) causing stem rot disease in groundnut isolated from soil. In dual culture technique the isolate GRT-1 was found more effective among the all isolates due to its complete over growth on S. rolfsii. Screened was done by the two intervals i.e fourth day and eighth day after inoculation. In dual culture, among ten Trichoderma spp. isolates tested, by eighth day, Trichoderma isolate GRT-1 showed over growth of 42.00 mm with sporulation on S. rolfsii (S.r-9) was found more effective among the entire test isolates due to its complete over growth on S. rolfsii (S.r-9)

Key words: *Trichoderma spp., Sclerotim rolfsii, Dual culture*

INTRODUCTION

Groundnut is grown in nearly 100 countries across the globe. It occupies 24.6 million ha worldwide with a total production of 41.3 million tonnes during 2012. In India, the total cultivated area of groundnut crop was 5.31million ha with production of 6.93 million tonne[2] and grown across the states Gujarat, Maharashtra, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Rajasthan, Karnatka and Madhya Pradesh. There are several soil-borne fungal diseases affecting yield of groundnut, among the all stem rot disease caused by *Sclerotium rolfsii* Sacc., a necrotropic soil borne fungus causes disease on wide range of agricultural and horticultural crops including groundnut. Yield losses usually range from 10 to 25% in India, about 20-60% of pod yield reduction was observed due to pod rot in widely cultivated varieties, JL 24, KRG 1, Dh 40, TMV 2 in Karnataka and Andhra Pradesh[1].

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Species of *Trichoderma* used as biological control agents against soil borne plant pathogenic fungi[9]. The advantage of using *Trichoderma* in managing soil borne plant pathogens are eco-friendly, effective, ease of mass culturing with less cost of production and growth promoting effect. Acts as biocontrol agents against plant pathogens based on various mechanisms such as the production of antifungal metabolites, competition for space and nutrients and mycoparasitism[7].

MATERIAL AND METHODS

Composite soil samples were collected from rhizosphere of healthy plants in stem rot infected groundnut field and shade dried Serial dilution technique[8] was used to isolate *Trichoderma* spp**.** from rhizosphere of groundnut. Antagonistic mycoflora were isolated on *Trichoderma* Selective Medium. One ml of final dilution of soil suspension was poured on to sterilized petri plates and then medium was poured at lukewarm stage. Plates were rotated gently to get uniform distribution of soil suspension in the medium. The plates were incubated at 28 $\pm$ 1^0C and observed at frequent intervals for the development of colonies. Three days old colonies of *Trichoderma* isolates were picked up and purified by single hyphal tip method. A total of ten *Trichoderma* spp. isolates were identification based on Mycological Keys described by Barnett *et al*[4]., and used for further studies.

Dual Culture Technique

Individual *Trichoderma* isolate was dual cultured with *S. rolfsii* isolate in *vitro*[11]. Twenty ml of melted and cooled PDA medium was poured into Petri plates and allowed to solidify. 5 mm culture disc of *Trichoderma* was placed 1cm away at one end of petri plate. A 5 mm test pathogen culture disc was placed 1cm away at the opposite end (with a gap of 7 cm between the two culture discs). Plates monocultured with either of the test fungi served as check. Three replications were maintained for each treatment.

The per cent inhibition of radial growth of the test *S. rolfsii* was calculated by using following formula.

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

Where,

I = Per cent reduction in growth of *S. rolfsii*
C = Radial growth (mm) in control
T = Radial growth (mm) in treatments

RESULTS AND DISCUSSION

At fourth day the per cent inhibition of *Trichoderma* spp. isolates were presented in the (Table 1). Among the 10 *Trichoderma* spp. isolates tested, GRT-8 isolate showed maximum percentage of inhibition (72.50%) followed by GRT-10 (70.75%) and GRT-3 (66.58%). The inhibition percentage of other isolates in descending order as GRT-7 (62.50%), GRT-5 (60.42%), GRT-1 (60.00%), GRT-9 (59.58%), GRT-2 (26.25%), GRT-4 (41.67%) and least per cent inhibited isolate was GRT-6 (23.75%).

Table 1: In *vitro* screening of *Trichoderma* isolates against *S. rolfsii* by dual culture technique at 4th day

S. No	Isolate	*S .rolfsii* mean growth (mm)	Per cent inhibition over control
1	GRT-1	32.00	60.00 (50.75)*
2	GTR-2	35.00	56.25 (48.57)
3	GRT-3	26.70	66.58 (54.68)
4	GRT-4	46.70	41.67 (40.18)
5	GRT-5	31.70	60.42 (50.99)
6	GRT-6	61.00	23.75 (29.13)
7	GRT-7	30.00	62.50 (52.21)
8	GRT-8	22.00	72.50 (58.34)
9	GRT-9	32.30	59.58 (50.66)
10	GRT-10	23.30	70.75 (57.24)
11	Control	100.00	00.00
	CD		4.53
	SE(m)		1.52
	SE(d)		2.15
	CV		5.36

* Values in parenthesis are angular transformed value

Among the all ten isolates of *Trichoderma*, over growth was observed in GRT-1 (17.00 mm), followed by GRT-2 (16.00 mm), GRT-5 (12.00 mm), GRT-7 (8.00 mm) and GRT-9 (6.00 mm) on *S. rolfsii* and also *S. rolfsii* (over growth on *Trichoderma* isolates of GRT-6 (19.40 mm) and GRT-4 (19.00 mm) (Table 2 & Fig 1) In interaction involving GRT-10 Vs *S. rolfsii*, GRT-8 Vs *S. rolfsii* and GRT-3 Vs *S. rolfsii* inhibition zone was measured as 5.70 mm, 3.00 mm and 2.00 mm respectively. However, In GRT-1 Vs *S. rolfsii* interactions GRT-1 could overcome maximum inhibitory effect of *S. rolfsii* within four days of incubation.

Table 2: Radial growths of *Trichoderma* spp. isolates and *S .rolfsii* in dual culture plates at 4th day

S. No	Isolate	*S. rolfsii* growth (mm)	*Tricoderma* growth (mm)	Over growth (mm) of *Trichoderma*	Over growth (mm) of *S. rolfsii*	Zone of inhibition (mm)
1	GRT-1	32.00	49.10	17.00	-	-
2	GTR-2	35.00	51.60	16.00	-	-
3	GRT-3	26.60	42.00	-	-	2.00
4	GRT-4	46.00	37.00	-	9.00	-
5	GRT-5	31.60	44.30	12.70	-	-
6	GRT-6	61.00	40.06	-	19.40	-
7	GRT-7	30.00	48.00	8.00	-	-
8	GRT-8	22.00	45.60	-	-	3.00
9	GRT-9	32.00	44.60	6.00	-	-
10	GRT-10	23.30	41.00	-	-	5.70
11	Control	80.00	80.00			

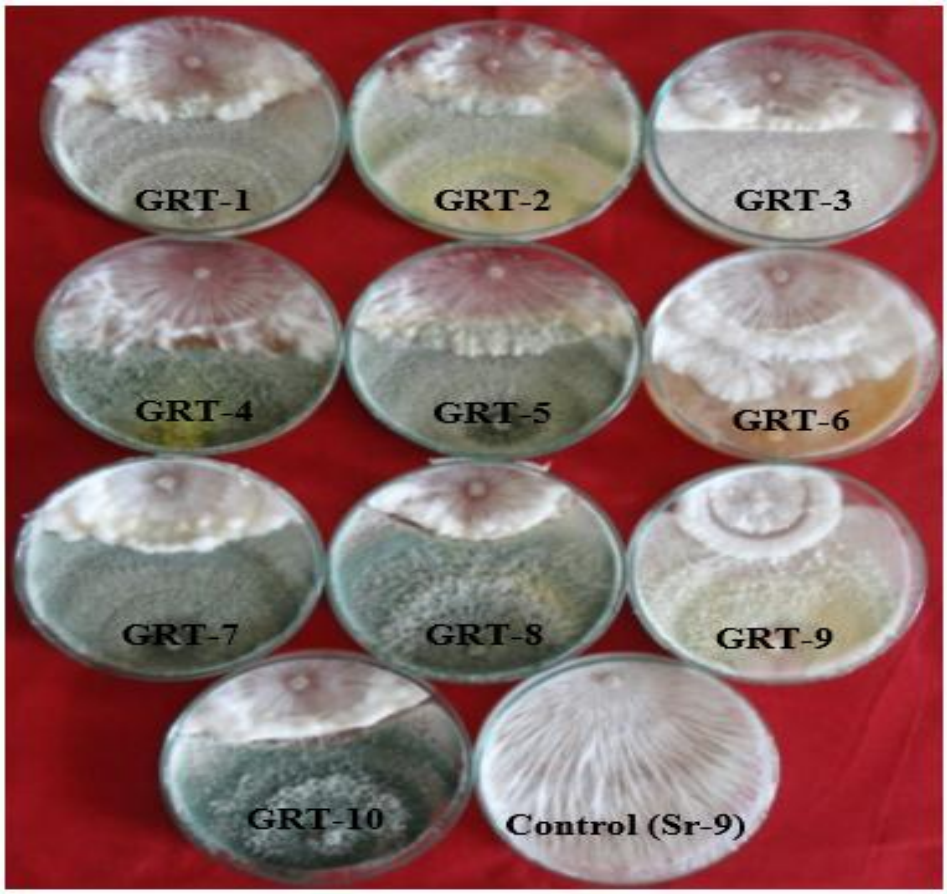

Fig. 1: *In vitro* interactions of *Trichoderma* spp. against *S. rolfsii* in dual culture at 4 days after incubation

By eight days, the radial growth of *S. rolfsii* and *Trichoderma* isolates were observed as different from the radial growths at fourth day (Fig 2). Among the *Trichoderma* isolates, GRT-1, followed by GRT-5, GRT-2 and GRT-7 showed over growth of 42.00 mm, 31.60 mm, 25.00 mm and 20.00 mm respectively on *S. rolfsii* (Table 3 & Fig 3), but the isolate GRT-1 showed maximum over growth with sporulation on *S. rolfsii* Also observed over growth of *S. rolfsii* on *Trichoderma* isolates of GRT-9 (54.60 mm), GRT- 6 (50.60 mm) and GRT-4 (28.00 mm) (Fig 4). It may be remembered have that interactions involving GRT-10 Vs *S. rolfsii*, GRT-8 Vs *S. rolfsii* and GRT-3 Vs *S. rolfsii* showed 5.70mm, 3.00mm and 2.00mm inhibition zone on eight days (Fig 5). However, the isolate GRT-1 was found more effective among the entire test isolates due to its complete over growth on *S. rolfsii*.

Table 3: Radial growths of *Trichoderma* spp. isolates and *S . rolfsii* in dual culture plates at 8th day

S. No	Isolate	*S. rolfsii* growth (mm)	*Tricoderma* growth (mm)	Over growth (mm) of *Trichoderma*	Over growth (mm) of *S. rolfsii*	Zone of inhibition (mm)
1	GRT-1	32.00	80.00	42.00	-	-
2	GTR-2	35.00	60.00	25.00	-	-
3	GRT-3	26.60	42.00	-	-	2.00
4	GRT-4	65.00	37.00	-	28.00	-
5	GRT-5	31.60	70.00	31.60	-	-
6	GRT-6	80.00	40.60	-	50.60	-
7	GRT-7	30.00	60.00	20.00	-	-
8	GRT-8	22.00	45.60	-	-	3.00
9	GRT-9	80.00	44.60	-	54.60	-
10	GRT-10	23.30	41.00	-	-	5.70
11	Control	80.00	80.00			

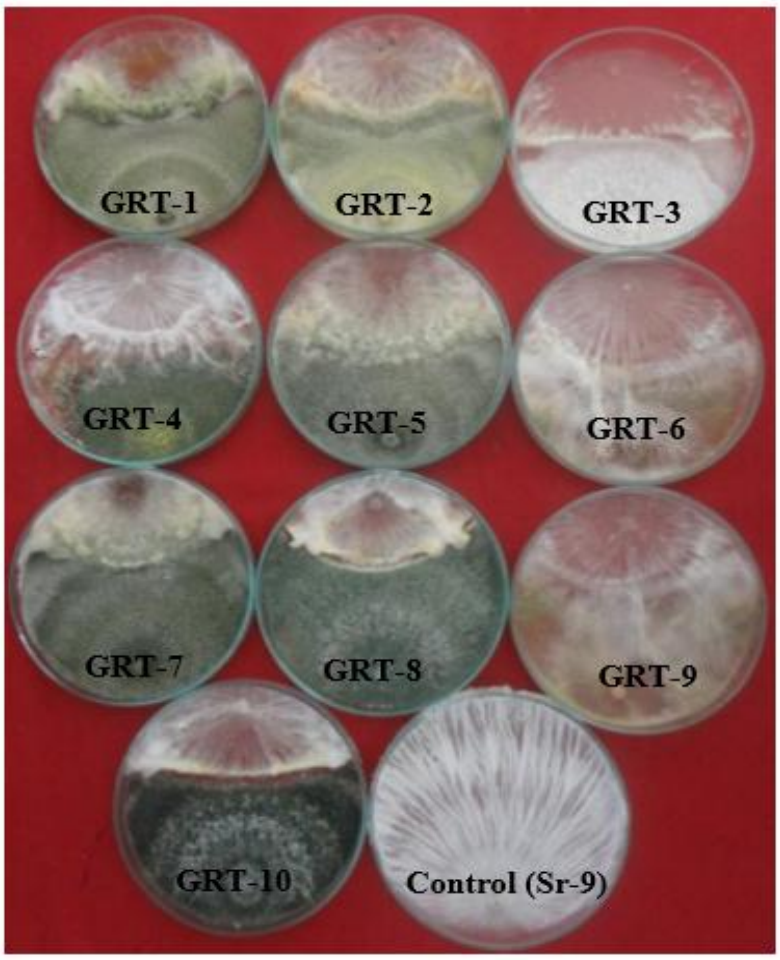

Fig 2: In vitro interactions *of S. rolfsii* Vs *Trichoderma* spp. at 8th day after incubation along with control

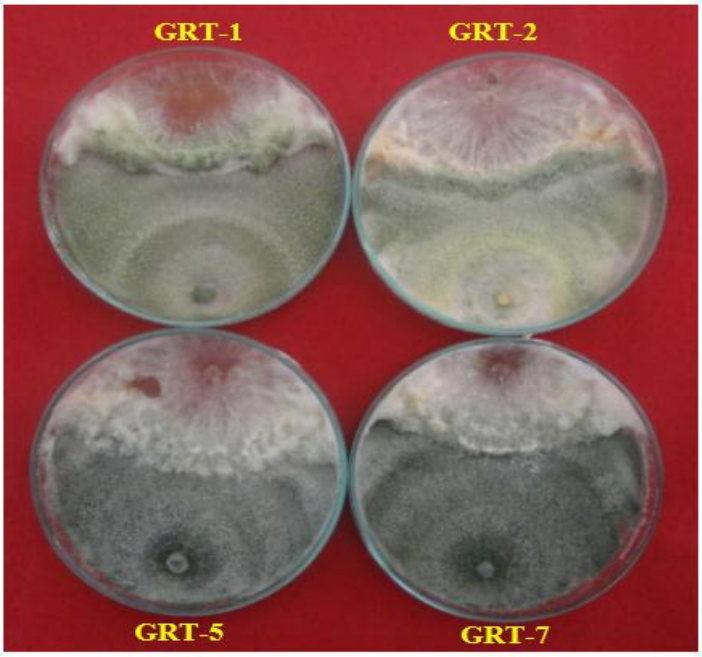

Fig. 3: Photograph showing over growth of *Trichoderma* spp. on *S. rolfsii*

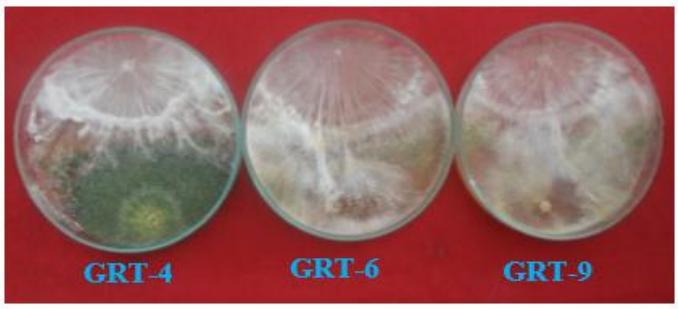

Fig. 4: Photograph showing over growth of *S. rolfsii* on *Trichoderma* spp. Isolates

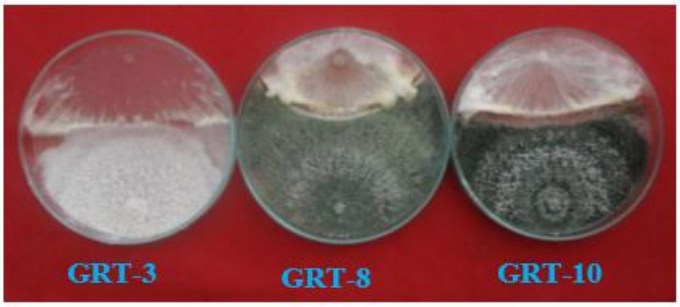

Fig. 5: Photograph showing zone of inhibition between the *S. rolfsi*i and *Trichoderma* spp. isolates

These results were supported with earlier workers. Rapid growth of *T. Harzianum* may give it an advantage in the competition with pathogenic fungi for space and nutrients[3]. The inhibition was supported by Elad *et al*[6]., who reported that *Trichoderma* spp. attached to the *S. rolfsii* either by hyphal coils, hooks, or appressoria. Lysed sites and penetration holes were found in hyphae of the pathogenic fungi, followingremoval of parasitic hyphae and high β-1,3glucanase and chitinase activities were detected in dual agar cultureswhen compared with fungus alone. Suppression of *Macrophomina phaseolina* by overgrowth of *Trichoderma* spp., colonies in the culture medium accompanied by hyphal coiling, hyphal abnormalities, reduction in sclerotial production and lysis of hyphae and sclerotia was reported[10]. Bhuiyan *et al*[5]., reported that, *T. harzianum* (TH)-18 showed the highest

(83.06%) reduction of the radial growth followed by TH-2 (74.19%).

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