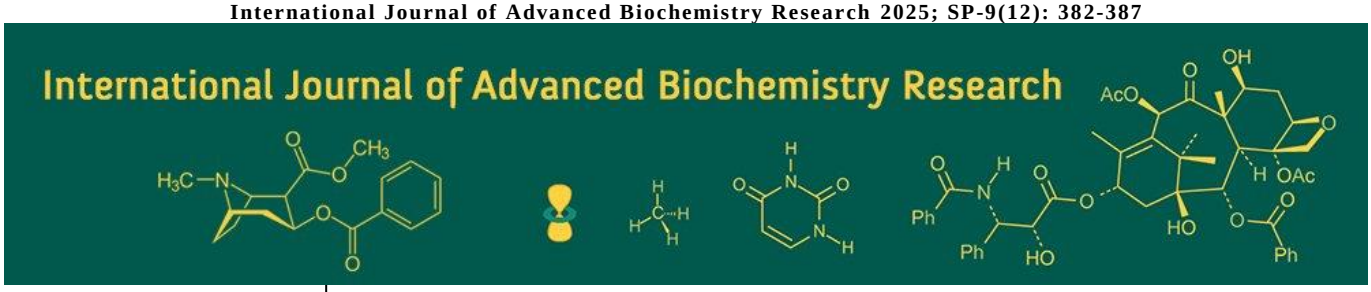

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In vitro evaluation of *Trichoderma* spp against collar rot pathogen of chickpea

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Abstract
Chickpea is a *rabi* crop, mainly grown in the areas of water scarcity which is attacked by various soil borne pathogens where collar rot disease caused by *Sclerotium rolfsii* Sacc is a major concern causing higher yield losses. To suppress the pathogen, naturally available *Trichoderma* spp were isolated from the chickpea growing areas of Andhra Pradesh and *in vitro* studies were performed to identify the antagonistic activity using thirteen *Trichoderma* isolates and best antagonist were identified based on qualitative parameters such as zone of inhibition, overgrowth potential, lysis and sporulation. Based on these observations T_2, T_3, T_5 and T_9 were identified as a potential suppressors for collar rot pathogen.

Key words: *Sclerotium rolfsii, Trichoderma, in vitro*

Introduction
Chickpea is the third most important legume in south Asia after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). India ranks first in area and production with 9.69 mha, 13.12 mt with a productivity of 1142 kgha^{-1} (Indiastat, 2021-2022) [9]. Major gram producing states in India are Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh which contribute to 90% area and 91% production in the country (Singh *et al.,* 2010) [19]. In Andhra Pradesh, it occupies an area of 0.459 million hectares with an annual production of 0.559 mt and productivity of 1218 kgha^{-1} during 2019-2020 (ICAR-AICRP on Chickpea PC report, 2020-21). Low productivity is mainly due to various biotic and abiotic factors. The major biotic factors include various pathogens, wilt complex pathogens (*Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*) cause 100% losses significantly under climate change situation (Pande *et al.,* 2010) [13]. To deter the growth of the S. *rolfsii* pathogen which accounts for 55-95% mortality of seedlings under heavy rainfall and high soil moisture (Sharma and Ghosh, 2017) [17]. High reproductive capacity, ability to survive under unfavourable conditions and hyper-parasitizing nature on phytopathogenic fungi lead to the success of *Trichoderma* species as a biocontrol agent (Howell, 2003) [8] *In-vitro* studies have been formulated to test their antagonistic efficacy using *Trichoderma*.

Material and Methods
Infected plants collected from chickpea infected fields Root bits of infected plant were cut, surface sterilized using 1% sodium hypochlorite for a minute and rinsed in three changes of distilled water to remove the disinfectant. Root bits wereblot dried (sterilized blotting paper) before transferringaseptically on to PDA plates and thenincubated at 27±1°C in an incubator. Two days old mycelial bits developed on diseased roots were aseptically transferred to glass slides and observations were made to confirm their identity based on morphological characters (mycelium, conidia and conidiophore).
Soil samples collected from the rhizosphere of chickpea growing areas were air dried for 24 hours and sieved through a 2mm sieve. Twenty milligrams of this sieved soil was transferred to a Petri plate and spread uniformly on *Trichoderma* selective medium (TSM) under aseptic conditions. The plates were incubated at 28°C for three to five days and the colonies were allowed to sporulate. Thus obtained *Trichoderma* cultures were sub-cultured on PDA after confirmation.

Thirteen *Trichoderma* isolates were used to test their antagonistic efficacy against *Sclerotium rolfsii* by dual culture technique (Dennis and Webster, 1971) [7]. Twenty ml of sterilized PDA was aseptically poured into 9.0 cm diameter Petri plates. Mycelial discs of 5 mm from actively growing three days old cultures of individual antagonist or the pathogen were cut in case of *Sclerotium rolfsii* were inoculated 7.0 cm apart (leaving 1.0 cm from the periphery). The plates inoculated with pathogen alone served as control. Inoculated plates were incubated at 27±1°C for 72 hrs. The per cent inhibition in growth was calculated by the formula

Table 1: List of *Trichoderma* isolates

S.No	*Trichoderma* isolates	Collected from
1	T_1	Bapatla
2	T_2	Throvagunta 1
3	T_3	Gundlapalli 1
4	T_4	Venkupalem 1
5	T_5	Gundreddipalem 1
6	T_6	Jangunguntla 1
7	T_7	Parchur 1
8	T_8	Parchur 2
9	T_9	Venkataramannagudem
10	T_{10}	Venkataramannagudem
11	T_{11}	Venkataramannagudem
12	T_{12}	Venkataramannagudem
13	T_{13}	Venkataramannagudem

$$\text{Per cent inhibition (\%)} = \frac{\text{Radial growth in control plate} - \text{Radial growth in dual culture plate}}{\text{Radial growth in control plate}} \times 100$$

Observations on radial growth of interacting test fungi, overgrowth, zone of inhibition, lysis, pigmentation, sporulation of *Trichoderma* spp. were recorded.

Results

S. *rolfsii* was identified by the growth of white mycelia threads along with small, uniformly sized, globoid sclerotia. Sclerotial bodies were initially white and turned to dark brown at maturity in accordance with Booth (1971) [5], In monoculture plates, radial growth of S. *rolfsii* recorded as 3.13, 6.80 and 7.20 of radius after 1, 3 and 5 days after inoculation respectively. Variation existed among S. *rolfsii* in interaction with various isolates of *Trichoderma*. Though there was no physical contact between the two interacting fungi up to 5 days after inoculation, significant reduction in radial growth of S. *rolfsii* was observed. The radial growth of S. *rolfsii* in dual culture plates ranged between 1.40-2.37, 2.17-4.30 and 2.57-5.03 at 1, 3 and 5 days after inoculation which is significantly lower than that in mono-cultured plate (3.13, 6.80 and 7.20 cm). Inhibition in the early stage reveals the effect of diffusible metabolites of antagonistic *Trichoderma* spp. tested against S. *rolfsii* which prevented further advancement of S. *rolfsii* in capturing the food source. In the present study, per cent inhibition in the growth of S. *rolfsii* isolates at one day after inoculation varied between 24.47 to 55.43%, 17.60 to 36.32% at 3 DAI and 11.97 to 32.36% at 5 DAI in dual culture plate. It was observed that isolate T3 exhibited highest per cent inhibition of 32.36 per cent of radial growth followed by isolate T_7, T_8 and T_9 recorded 31.68, 31.43 & 31.06 per cent inhibition of radial growth and which were on par with each other against S. *rolfsii*. Isolate T_2 was found to be effective with 30.76 per cent inhibition of radial growth and least per cent inhibition of 11.97 was exhibited by isolate T_4. (Table 2, Fig 1).

It was observed that the reduction in per cent inhibition in the growth of S. *rolfsii* indicated that the radial growth of S. *rolfsii* did not cease in dual culture plates. Initial observation at 1 day after inoculation revealed a gap of 0.76-3.20 cm between the *Trichoderma* isolates and the test pathogen in dual cultured plates i.e., no physical contact was observed between both fungi. In T_{11}, T_{12} isolates no gap was found indicating the presence of physical contact between test antagonists and test pathogen at 3 DAI. Continued incubation up to 5 days after inoculation revealed further decrease in gap in the dual culture plates. In T_4, T_5, T_{11} and T_{12} isolates, no gap exists which indicating that the fungi in dual culture plate were in physical contact with each other (Table 3).

Radial growth of S. *rolfsii* was converted into growth rate (mm h-1) at 1, 3 and 5 days after inoculation and presented in the Table 4.11. Progressive mean growth rate of S. *rolfsii* was observed upto 5 days, it was recorded a mean growth rate of 0.83 mm h-1 at 1 DAI and further decreased at 3 and 5 DAI @ 0.19 and 0.08 mm h-1 respectively. In the present investigation, it was observed that S. *rolfsii* growth rate was highly decreased after 3 DAI compared to 1 DAI indicating less sensitivity of test S. *rolfsii* isolate to all test *Trichoderma* isolates. Mean growth rate of *Trichoderma* isolates was recorded (1.15 mm h-1) at 1 day after inoculation and further decreased at 3 and 5 DAI @ 0.17 and 0.04 mm h-1 respectively (Table 4).

The radial growth of *Trichoderma* isolates in dual culture plates ranged between 1.87-3.87, 2.47-5.07, 3.00-4.80 cm at 1, 3 and 5 days after inoculation which is significantly lower than that in mono-cultured plate (2.20-4.20, 5.30-9.00 and 7.90-9.00 cm). Per cent inhibition in the growth of *Trichoderma* isolates at one day after inoculation varied between 0.00 to 52.01%, 25.00 to 68.35% at 3 DAI and 46.67 to 66.33% at 5 DAI in dual culture plate (Table 5, Fig 3).

In order to study the antagonistic effects on one another in dual cultured plates, observations were also recorded on zone of inhibition, lysis of interacting fungi, sporulation, pigmentation etc.

The present investigation also revealed that incubation up to five days was insufficient to categorize an isolate as

potential or not *in vitro*. Hence, other qualitative parameters such as zone of inhibition, overgrowth potential, lysis, sporulation in *Trichoderma* at Zi and pigmentation in S. *rolfsii* were also considered for screening and selecting potential *Trichoderma* isolates (Table 6, Plate 2). Accordingly, the following groups were formed.

1. Either Zi or lysis of S. *rolfsii* or both but without overgrowth of *Trichoderma* on S. *rolfsii*: The *Trichoderma* isolates *viz*., T_6, T_8, T_3, T_2, T_1, T_7, T_9 and T_{10} isolates were considered least potential (Table 6).
2. Either Zi or lysis of S. *rolfsii* or both, overgrowth of *Trichoderma* on S. *rolfsii*, sporulation in *Trichoderma* not inhibited, with or without change in S. *rolfsii* medium pigmentation, faster radial growth in monoculture: *Trichoderma* isolates T_3, T_2 and T_9 isolates were considered as having high antagonistic potential against S. *rolfsii* (Table 6).
3. T_5, T_2, T_3 and T_9 isolates were selected which were fast in growth, per cent inhibition, lysing, sporulation, overgrowing the S. *rolfsii* (Table 6, Fig 2).

Discussion: Rolfs (1892) [16] first identified the collar rot fungus and Saccardo (1911) [1] named the fungus as *Sclerotium rolfsii*. Curzi (1931) [6] proposed Corticum *rolfsii* Curzi as the perfect state of S.*rolfsii*. Subramanian (1964) [20] reported that mycelium was septate, much branched, silky or cottony white, spreading in strands of fan like appearance. Sclerotia on germination produced two forms of mycelial growth i.e., primary hyphal mycelium produced from individual strands on the sclerotial surface and secondly, eruptive mycelium characterized by aggregates of mycelium, bursting through sclerotial rind (Punja, 1985) [15].

Collar rot caused by *Sclerotium rolfsii* survives in the infected tissues and plant debris as sclerotial bodies and usually attacks the collar region (Ayacock, 1966) [3]. It is an emerging soil borne disease that may incite 55 to 95 percent mortality of seedlings under favourable conditions like heavy rainfall and high soil temperature (Sharma and Ghosh, 2017) [17].

Sclerotium rolfsii is an unpredictable pathogen because of its soil borne nature and wide host range (Singh and Singh, 1991) [18]. Patil and Rane (1985) [14] reported that as the age of the plant increased the infection of S. *rolfsii* decreased. The pathogen has an extensive host range of atleast 500 species in 100 families.

Weindling (1932) [24] was the first person to demonstrate the *Trichoderma* spp, parasitized as well as inhibited the development of S. *rolfsii*, including the potential of these fungi as biocontrol agents.

Mathur and Sarbhoy (1978) [10] evaluated the biocontrol efficacy of T. harzianum and T. viride *in vitro* and in pot culture against S. *rolfsii*. Isolates of T. viride and T. harzianum inhibited mycelial growth of the pathogen by 88 and 86 per cent respectively, in dual culture. Under glass house experiments, use of these biocontrol agents could cause only 20 and 13 per cent infection against 100 per cent in untreated controls.

Bell *et al.* (1982) [4] tested the antagonistic activity of several *Trichoderma* isolates against S.*rolfsii* and found that only 12 per cent of the isolates completely overgrew the pathogen. Viability of S. *rolfsii* was microscopically observed nine days after interaction and the complete disintegration of sclerotia was observed in 12% of the isolates.

Sclerotium rolfsii has an extensive host range and is widely distributed in tropical and subtropical regions of the world. It is common in India, USA, Africa, Australia and countries surrounding Mediterranean region (Ayacock, 1966 and Punja, 1985) [3, 15]. The pathogen rarely occurs when temperature falls below 0ºC.High soil moisture and relatively high atmospheric temperature (25-30ºC) accelerate disease development and under favourable environmental conditions root rot incidence up to 55 per cent has been observed in India (Waraitch *et al.*, 1986) [23].

Upadhyay and Mukhopadhyay (1986) [22] reported that an isolate of T. harzianum directly attacked and lysed the mycelium and sclerotia of the pathogen, S.*rolfsii* in dual culture. Hyphal coiling, entry through haustoria like structures and direct entry into the hyphae and sclerotia were observed. The collar rot pathogen, S. *rolfsii* attacks the seedlings and grown up plants. The first sign of infection are dark brown lesions on the stem or just beneath the soil level. The first visible symptoms are progressive yellowing and wilting of the leaves followed by white, fluffy mycelium on the infected tissues (Nene *et al.* 1991) [12].

Muthamilan and Jeyarajan (1992) [11] studied the *in vitro* effect of eight isolates of antagonistic fungi on S.*rolfsii* causing root rot of groundnut and reported that T.viride was superior to all other isolates in arresting the mycelial growth and the sclerotial production of the pathogen.

It was noticed that the symptoms of chickpea wilt complex appear at different growth stages of crop. Up to 30 DAS, *Sclerotium rolfsii* causing collar rot was noticed; 30-60 DAS, Fusarium wilt caused by Fusariumoxysporum f. sp. ciceri was observed. At the end of the crop season i.e., at 60 DAS, Rhizoctonia dry root rot caused by Macrophomina phaseolina was observed (Swathi, 2015 and Amulya, 2021) [2, 21].

Table 2: Bioefficacy of *Trichoderma* isolates against radial growth of *Sclerotium rolfsii in vitro*.

S. No	*Trichoderma* isolates	1 DAI		3 DAI		5 DAI	
		Radial growth of *S. rolfsii* (cm)	Inhibition%	Radial growth of *S. rolfsii* (cm)	Inhibition%	Radial growth of *S. rolfsii* (cm)	Inhibition%
1	T_1	2.07(8.26)^{c}	34.04	2.97 (9.91)^{de}	28.69	3.33 (10.51)^{f}	25.39
2	T_2	1.77(7.63)^{b}	43.62	2.17 (8.45)^{a}	36.32	2.73 (9.51)^{abc}	30.76
3	T_3	1.84(7.79)^{b}	41.28	2.50 (9.09)^{b}	31.13	2.57 (9.20)^{a}	32.36
4	T_4	2.10(8.33)^{c}	43.83	4.30 (11.95)^{f}	17.60	5.03 (12.96)^{g}	11.97
5	T_5	2.20(8.53)^{de}	29.79	2.70 (9.45)^{bcd}	31.13	2.87 (9.74)^{bcd}	29.53
6	T_6	1.40(6.78)^{a}	55.43	2.73 (9.51)^{bcde}	30.83	2.87 (9.74)^{bcd}	29.52
7	T_7	2.17(8.46)^{cd}	30.85	2.60 (9.27)^{bc}	32.06	2.63 (9.33)^{ab}	31.68
8	T_8	1.77(7.63)^{b}	43.62	2.63 (9.33)^{bc}	31.75	2.67 (9.38)^{ab}	31.43
9	T_9	1.77(7.63)^{b}	43.62	2.67 (9.39)^{bcd}	31.44	2.70 (9.45)^{ab}	31.06
10	T_{10}	2.13(8.39)^{cd}	31.91	2.97 (9.90)^{de}	28.72	3.30 (10.46)^{ef}	25.68
11	T_{11}	2.33(8.78)^{ef}	25.53	3.03 (10.02)^{e}	28.10	3.00 (9.97)^{cde}	28.32

12	T_{12}	2.37(8.84)^{f}	24.47	2.97 (9.90)^{de}	28.75	3.17 (10.25)^{ef}	26.84
13	T_{13}	2.07(8.26)^{c}	34.04	2.80 (9.63)^{bcde}	30.20	3.10 (10.13)^{def}	27.44
Control		3.13(10.19)^{g}		6.80 (15.10)^{g}		7.20 (15.56)^{h}	
SEm (±)		0.09		0.18		0.17	
C.D (%)		0.26		0.53		0.50	
C.V		1.90		3.18		2.90	

Each treatment was replicated thrice. DAI - Days after inoculation. Values with similar alphabets do not differ significantly
* Values in the parenthesis are arc sine transformed values

Table 3. Relative radial growth of *Trichoderma* isolates and S. *rolfsii* in dual culture

S. No	*Trichoderma* isolates	1 DAI				3 DAI				5 DAI			
		Trichoderma (cm)	*S. rolfsii* (cm)	Total (cm)	zi (cm)	*Trichoderma* (cm)	*S. rolfsii* (cm)	Total (cm)	zi (cm)	*Trichoderma* (cm)	*S. rolfsii* (cm)	Total (cm)	zi (cm)
1	T_1	2.93	2.07	5.00	2.00	3.07	2.97	6.04	0.96	3.20	3.33	6.53	1.47
2	T_2	2.03	1.77	3.80	3.20	2.70	2.17	4.87	2.13	3.03	2.73	5.76	1.24
3	T_3	3.10	1.84	4.94	2.06	3.80	2.70	6.50	0.50	3.80	2.57	6.37	0.63
4	T_4	1.87	2.10	3.97	3.03	2.47	4.30	6.77	0.23	4.20	5.03	9.23	0.00
5	T_5	3.10	2.20	5.30	1.70	4.10	2.70	6.80	0.20	4.20	2.87	7.07	0.00
6	T_6	2.97	1.40	4.37	2.63	3.67	2.73	6.40	0.60	3.67	2.87	6.54	0.46
7	T_7	2.63	2.17	4.80	2.20	3.27	2.60	5.87	1.13	3.33	2.63	5.96	1.04
8	T_8	2.13	1.77	3.90	3.10	3.73	2.63	6.36	0.64	3.73	2.67	6.40	1.30
9	T_9	2.97	1.77	4.74	2.26	3.87	2.67	6.54	0.46	3.90	2.70	6.60	0.40
10	T_{10}	2.73	2.13	4.86	2.14	3.03	2.97	6.00	1.00	3.00	3.30	6.30	0.70
11	T_{11}	2.80	2.33	5.13	1.87	4.07	3.03	7.10	0.00	4.07	3.00	7.07	0.00
12	T_{12}	3.87	2.37	6.24	0.76	4.77	2.97	7.74	0.00	4.80	3.17	7.97	0.00
13	T_{13}	2.83	2.07	4.90	2.00	3.77	2.80	6.57	0.43	3.77	3.10	6.87	0.13

Table 4: Growth rate of *Trichoderma* and S. *Rolfsii* isolates in dual culture plates

S. No	*Trichoderma* isolates	Growth rate of *S. rolfsii* (mm h^{-1})			Growth rate of *Trichoderma* (mm h^{-1})		
		1 DAI	3 DAI	5 DAI	1 DAI	3 DAI	5 DAI
1	T_1	0.86	0.19	0.08	1.22	0.03	0.03
2	T_2	0.74	0.18	0.12	0.85	0.14	0.07
3	T_3	0.77	0.18	0.48	1.29	0.14	0.00
4	T_4	0.88	0.46	0.15	0.78	0.13	0.36
5	T_5	0.92	0.10	0.03	1.29	0.21	0.02
6	T_6	0.58	0.28	0.03	1.24	0.15	0.00
7	T_7	0.90	0.09	0.01	1.10	0.13	0.01
8	T_8	0.74	0.18	0.01	0.89	0.33	0.00
9	T_9	0.74	0.19	0.01	1.24	0.19	0.01
10	T_{10}	0.89	0.17	0.07	1.14	0.06	0.06
11	T_{11}	0.97	0.15	0.01	1.16	0.26	0.00
12	T_{12}	0.99	0.12	0.04	1.61	0.19	0.01
13	T_{13}	0.86	0.15	0.06	1.17	0.20	0.00
Mean		0.83	0.19	0.08	1.15	0.17	0.04

Table 5: Radial growth of *Trichoderma* against S. *rolfsii*

Radial growth of *Trichoderma* in dual cultured plates								Radial growth of *Trichoderma* in mono cultured plates			
S. No	*Trichoderma* isolates	1 DAI		3 DAI		5 DAI		S. No	1 DAI (cm)	3 DAI (cm)	5 DAI (cm)
		Radial growth (cm)	Inhibition %	Radial growth (cm)	Inhibition %	Radial growth (cm)	Inhibition %				
1	T_1	2.93(9.86)	1.35	5.07(13.00)	41.63	3.20(2.05)	59.65	1	3.0	5.3	7.9
2	T_2	2.03(8.19)	52.01	2.70(9.45)	68.35	3.03(2.01)	66.33	2	4.2	8.5	9.0
3	T_3	3.10(10.14)	0.00	4.07(11.24)	49.33	3.80(2.19)	57.78	3	3.1	7.5	9.0
4	T_4	1.87(7.84)	15.00	2.47(9.03)	64.05	4.20(2.28)	48.59	4	2.2	6.9	8.2
5	T_5	3.10(10.13)	8.82	4.90(11.68)	39.97	4.20(2.28)	48.53	5	3.4	6.8	8.2
6	T_6	2.97(9.91)	16.81	3.67(11.03)	59.22	3.63(2.15)	59.22	6	3.6	9.0	9.0
7	T_7	2.63(9.33)	20.30	3.27(10.08)	56.40	3.33(2.08)	63.00	7	3.3	7.5	9.0
8	T_8	2.13(8.39)	33.44	4.73(11.14)	48.41	3.03(2.01)	56.63	8	3.2	7.2	8.6
9	T_9	2.97(9.80)	4.19	3.87(11.33)	47.70	3.90(2.21)	55.83	9	3.1	7.4	8.8
10	T_{10}	2.73(9.45)	5.86	3.03(10.02)	52.43	3.00(2.00)	63.01	10	2.9	6.4	8.1
11	T_{11}	2.80(9.63)	0.00	4.07(11.63)	34.67	5.07(2.46)	53.91	11	2.8	6.2	8.8
12	T_{12}	3.87(9.75)	1.03	4.77(12.60)	25.00	4.80(2.41)	46.67	12	2.9	6.4	9.0

13	T_{13}	2.83(9.68)	2.41	3.77(11.19)	34.21	3.77(2.18)	52.88	13	2.9	5.7	8.0
SEm(±)		0.10		0.12		0.01					
C.D(P≤ 0.05)		0.30		0.37		0.03					
CV (%)		1.95		1.98		1.02					

Table 6: Selection of potential *Trichoderma* isolates against S. *rolfsii in vitro* based on qualitative characters (5 DAI)

S. No.	*Trichoderma* isolates	Zone of inhibition (Zi)	Lysis		Sporulation in *Trichoderma* at Zi	Overgrowth of *Trichoderma* on *S. rolfsii*	Pigmentation in *S. rolfsii*	Growth of *Trichoderma* in mono culture plates	Growth of *Trichoderma* in dual culture plates	Total
			T	*S. rolfsii*						
1	T_1	2	-2	2	0	-2	2	4	2	8
2	T_2	2	0	2	2	-2	2	4	2	12
3	T_3	2	0	2	2	-2	2	4	2	12
4	T_4	0	0	0	0	-2	2	4	3	7
5	T_5	0	0	2	2	2	2	4	3	15
6	T_6	2	-2	2	0	-2	2	4	2	8
7	T_7	2	-2	2	0	-2	2	4	2	8
8	T_8	2	-2	2	0	-2	2	4	2	8
9	T_9	2	0	2	2	-2	2	4	2	12
10	T_{10}	2	0	2	0	-2	2	4	2	10
11	T_{11}	0	0	2	0	-2	2	4	3	9
12	T_{12}	0	0	2	0	-2	2	4	3	9
13	T_{13}	2	-2	2	0	-2	2	4	2	8

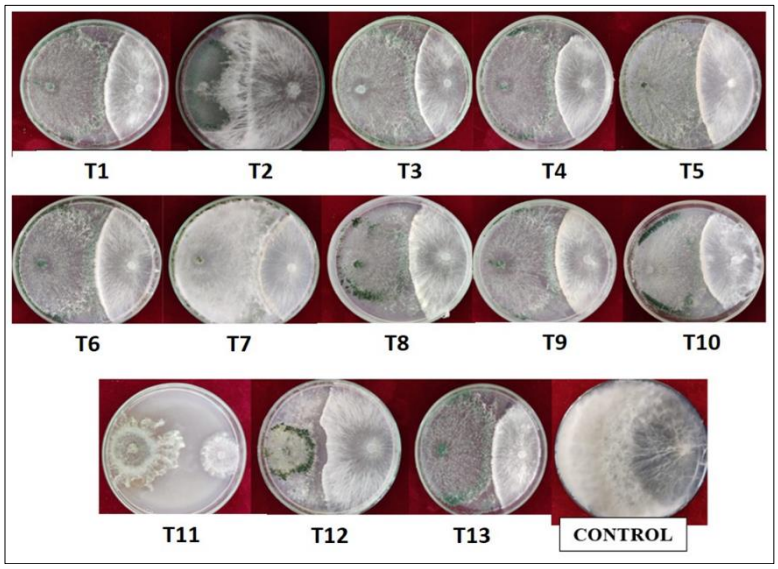

Fig 1: Dual culture of *Trichoderma* isolates against S. *rolfsii in vitro*

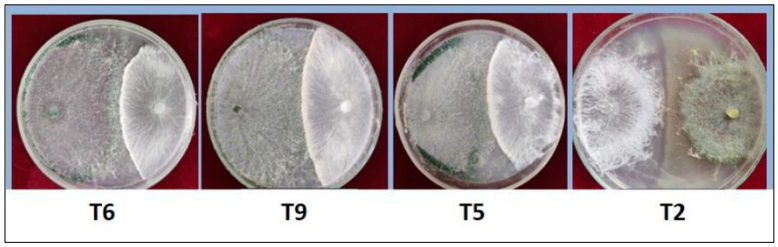

Fig 2: Effective *Trichoderma* isolates against S. *rolfsii*

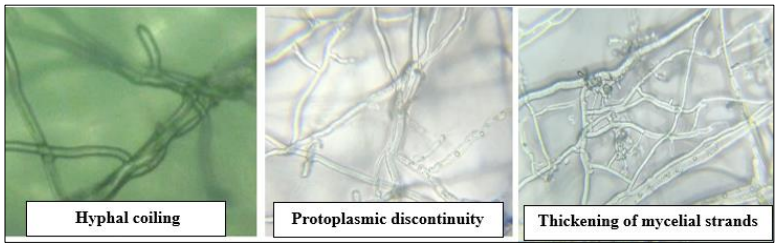

Fig 3: Mycoparasitism of *Trichoderma* on S. *rolfsii* at 400X

Conclusion

The present study identified the potential fungal antagonist when S. *Rolfsii* was dual cultured with *Trichoderma* isolates, T_3 exhibited highest per cent inhibition of 32.36 per cent of radial growth followed by isolate T_7, T_8 and T_9 recorded 31.68, 31.43 & 31.06 per cent inhibition of radial growth and which were on par with each other against S. *rolfsii*. Isolate T_2 was found to be next best effective with 30.76 per cent inhibition of radial growth and least per cent inhibition of 11.97 was exhibited by isolate T_4. In T_4, T_5, T_{11} and T_{12} isolates, no gap exists which indicating that the fungi in dual culture plate were in physical contact with each other.

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