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EFFECT OF VOLATILE METABOLITES RELEASED BY DIFFERENT *TRICHODERMA* SPP ON THE GROWTH OF PATHOGEN *SCLEROTIUM ROLFSII* AND EFFECT ON SCLEROTIA GERMINATION

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ABSTRACT
Trichoderma species are very well known natural gifted antagonist as well as growth promoter. Thirty potential isolates including *Trichoderma harzianum* (10), *T. viride*(12), *T. hamatum*(3),*T. reesei*(3),*T. piluliferum*(1) and *T. virens* (*Gliocladium deliquence*) (1) collected from different lentil growing areas from Bihar were screened *in vitro* for their efficiency through volatile metabolites in inhibiting the growth of *Sclerotium rolfsii*. The volatile metabolites released by antagonist of different period showed varying results of inhibition of growth of *S. rolfsii*. Among the (10) isolates of *T. harzianum* only the native T18 isolate of *T. harzianum* was found to provide 89.02% inhibition. Isolate T20 of *T. harzianum* showed 35.29% inhibition of the pathogen, rest of the *T. harzianum* had no effect. In case of *T. viride* out of 12 only isolates T11 and T26 restricted the growth by 17.65 and 50.59%. Isolate *T. virens* T30 was found to provide only 11.76% inhibition of the pathogen. All other *Trichoderma* spp. isolates had no perceptible effect on the pathogen's growth. At the end of 12 days of inoculation with the pathogen, mycelial degradation and lysis was evident in case of *T. harzianum* T18 isolate. Even Microscopic examination revealed that there was partial to complete mycelium degradation of the pathogen by the antagonist *T. harziaum* T18.

INTRODUCTION

Trichoderma are free living natural gifted bioagents as well as growth promoters which are highly interactive in the rhizosphere and foliar environments. *Trichoderma* have created ecofriendly, safe and non-chemical disease management system which have great importance in organic agriculture. *Trichoderma*, a soilborne myco parasitic fungus has been shown effective against many soil borne phytopathogens (Papavizas,1985; Harman *et al*., 1998; Dolatabadi *et al*., 2012). Biological control of soil borne phytopathogens has been the subject to extensive research in the last few decades. However, with the increasing interest in biological control, owing to environmental and economical concerns, thousands of research experiments are going on for searching novel, potential, safe and have ability to inhibit wide range of soilborne pathogens. *Trichoderma* spp is well documented as effective biological control agents of soil borne diseases which inhibit the pathogens by direct antagonism or by secreting several cell wall degrading enzymes, antibiotics (Sivan *et al*., 1984 and Coley-Smith *et al*.,1991). Many reports indicated that the application of *T. viride* and *T. harzianum* were found to be highly antagonistic to *S. rolfsii* and successful management of diseases in vegetables and legumes (Mathur and Sarbhoy,1978; Chet *et al*.,1979; Kamala and Devi, 2012).

Biological control of soil borne plant pathogens can be achieved successfully by seed coating, furrow application and root dip of seedlings with antagonists. Elad *et al*. (1980) have reported that the application of *T. harzianum* with wheat bran colonized rapidly in the soil and inhibits the infestation of *R. solani* and *S. rolfsii* in beans.

Sclerotium rolfsii Sacc., {teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough} and *Rhizoctonia solani*. Kuhn. {teleomorph= *Thanatephorus cucumeris* Frank (Donk)} are important soil borne pathogens which are very common in tropical, subtropical and temperate regions of the world. Both the pathogens are survives in the form of vegetative mycelium or sclerotia and causes several disease in crops plants and infected more than 500 species of cultivated and wild plants (Punja, 1985; Yaqub and Shahzad 2011; Sangle 2011; Sangle *et al*., 2012).

This idea emphasized the role of volatile metabolites secreted by *Trichoderma* spp and need for identifying effective isolate specifically management of potent sclerotial fungi like *S. rolfsii* in lentil growing areas of Eastern region of India.

MATERIALS AND METHODS

Isolation of the pathogen

The Lentil plants initially after 30-35 days after sowing (DAS) root rotted disease plants were collected from the Experimental farm of ICAR Research complex for Eastern Region Patna and farmers field in the month of end November in Rabi season. Small bits of the infected tissue were surface sterilized by dipping in 0.1% $HgCl_2$ solution for 30 second followed by

four subsequent washing with sterilized distilled water and then after kept of the bits in aseptic condition on Potato Dextrose Agar (PDA) at 25 + 1ºC. The mycelium hyphae converted mustard like numerous white to brown colored sclerotia which were then transferred on PDA for pure culture. These sclerotia are the resting fruiting bodies of the pathogens which serve as primary sources of inoculums which germinate by producing mycelium which radiate from the sclerotia and reached to the color region of the plants and cause infection. The cultural and morphological characteristic of the isolate were studied to identify the fungus associated with the root rot disease in lentil .

Isolation of the antagonist

Thirty isolates of *Trichoderma* belonging to *T. harzianum*, *T. viride*, *T. reesei*, *T. hamatum, T. piluliferum* and *T. virens* were collected and isolated from different lentil growing places in Bihar. The soil samples were collected from rhizosphere of different host plants adjacent to or between two diseased plants. The antagonist *Trichoderma* were then isolated using serial dilution plate techniques (Sangle and Bambawale, 2004) on Martin's Rose-Bengal Agar Medium (Martin, 1950). The isolated antagonists were purified by single spore hyphal tip method (Rangaswami, 1958). The various isolates were identified based on their morphological characters described by Rifai (1969) and later by Nagamani *et al*. (2002). The purified and identified cultures of *Trichoderma* spp. were maintained on PDA by sub-culturing at two months interval. Confirmations of *Trichoderma* spp were evaluated in laboratory for effect of volatile by dual culture against *S. rolfsii*.

Screening of volatile metabolites released by antagonists on the growth of *S. rolfsii*

Assay for volatile metabolites of *Trichoderma* spp Productions of volatile metabolites by *Trichoderma* spp were assayed as described by Dennis and Webster (1971a) with slight modifications. The *Trichoderma* isolates were centrally inoculated by placing 3 mm discs taken from 3 days old culture on PDA plates and incubated at 25 ± 1ºC for 3 days. The top of each petridish was replaced with bottom of the PDA plate inoculated centrally with the pathogen isolates. Petridishes with PDA medium without *Trichoderma* spp. at the lower lid and inoculated with pathogen maintained as control. Three replications were maintained for each treatment. The pairs of each petridish were sealed together with paraffin adhesive tape and incubated at 25 ± 1ºC. Colony diameter of the pathogen was measured at 3, 6 and 12 days after incubation and the inhibition of mycelial growth was calculated using the following formula.

Percent growth inhibition (I) = C – T/C X100

Where; I = Percent growth inhibition, C = Colony diameter of pathogen in control

T = Colony diameter /radial growth of pathogen in treatment

RESULTS AND DISCUSSION

Assay for volatile metabolites of *Trichoderma* spp

Thirty fungal bioagents antagonist were evaluated against *S. rolfsii*. It is very interesting observation were made in different

Table 1 : Effect of volatile metabolites of different *Trichoderma* spp on radial growth of *Sclerotium rolfsii*

Isolates	Per cent inhibition Days after inoculation (DAI)							
	2		6		12		Overall	
	Mean	+ SD	Mean	+ SD	Mean	+ SD	Mean	SD
1. T. harzianum	*40.16* ^{c}	*2.46*	*20.78* ^{cde}	*3.34*	*0.00* ^{e}	*0.00*	*20.31*	*17.52*
2. *T. harzianum*	31.10 ^{cde}	12.29	18.24 ^{cdef}	11.90	0.00 ^{e}	0.00	16.45	16.01
3. *T. viride*	-4.33 ^{jklm}	6.82	0.20 ^{f}	0.34	0.00 ^{e}	0.00	-1.38	4.07
4. *T. viride*	-5.12 ^{klm}	11.27	2.75 ^{ef}	2.45	0.00 ^{e}	0.00	-0.79	6.72
5. *T. viride*	17.72 ^{efgh}	8.71	8.24 ^{def}	9.63	0.00 ^{e}	0.00	8.65	10.05
6. *T. viride*	-4.72 ^{klm}	9.83	1.57 ^{f}	2.72	0.00 ^{e}	0.00	-1.05	5.84
7. T. hamatum	*13.39* ^{efghij}	*5.33*	*31.57* ^{c}	*27.47*	*0.00* ^{e}	*0.00*	*14.98*	*19.60*
8. *T. viride*	-2.76 ^{ijklm}	4.26	0.00 ^{f}	0.00	0.00 ^{e}	0.00	-0.92	2.54
9. *T. viride*	7.87 ^{ghijkl}	0.00	7.65 ^{def}	6.63	0.00 ^{e}	0.00	5.17	5.10
10. *T. harzianum*	12.60 ^{fghijk}	7.18	10.98 ^{def}	1.36	0.00 ^{e}	0.00	7.86	6.97

Table 1 : Cont....

Isolate	Per cent inhibition Days after inoculation (DAI)							
	2		6		12		Overall	
	Mean	+ SD	Mean	+ SD	Mean	+ SD	Mean	SD
11. *T. viride*	5.91 ^{ghijkl}	15.19	18.04 ^{cdef}	32.78	17.65 ^{d}	30.57	13.86	24.4
12. *T. hamatum*	-4.72 ^{klm}	2.46	1.76 ^{ef}	1.76	0.00 ^{e}	0	-0.99	3.28
13. *T. viride*	5.51 ^{ghijkl}	9.67	8.43 ^{def}	4.34	0.00 ^{e}	0	4.65	6.47
14. *T. reesei*	37.01 ^{cd}	13.01	16.08 ^{cdef}	12.91	0.00 ^{e}	0	17.7	18.5
15. *T. viride*	-4.33 ^{jklm}	4.77	0.00 ^{f}	0	0.00 ^{e}	0	-1.44	3.22
16. *T. hamatum*	-15.35 ^{m}	5.94	-0.59 ^{f}	1.02	0.00 ^{e}	0	-5.31	8.12
17. *T. harzianum*	6.69 ^{ghijkl}	11.99	2.16 ^{ef}	1.36	0.00 ^{e}	0	2.95	6.72
18. *T. harzianum*	84.25 ^{a}	4.92	100.00 ^{a}	0	89.02 ^{a}	0.68	91.09	7.42
19. *T. reesei*	43.31 ^{bc}	25.41	26.08 ^{cd}	20.46	0.00 ^{e}	0	23.13	24.95
20. *T. harzianum*	60.63 ^{b}	24.59	72.75 ^{b}	33.97	35.29 ^{c}	0	56.22	26.71

Table1 : Cont.....

Isolate	Per cent inhibition Days after inoculation (DAI) 2 Mean	+ SD	6 Mean	+ SD	12 Mean	+ SD	Overall Mean	SD
21. *T. harzianum*	19.29 ^{defg}	4.15	11.76 ^{def}	4.12	0.00 ^{e}	0	10.35	8.91
22. *T. harzianum*	0.39 ^{hijklm}	10.32	0.00 ^{f}	0	0.00 ^{e}	0	0.13	5.16
23. *T. harzianum*	11.81 ^{fghijk}	4.15	12.94 ^{cdef}	1.18	0.00 ^{e}	0	8.25	6.57
24. *T. reesei*	-6.69 ^{lm}	6.51	1.96 ^{ef}	1.8	0.00 ^{e}	0	-1.58	5.18
25. *T. harzianum*	18.90 ^{efg}	6.06	1.96 ^{ef}	1.7	0.00 ^{e}	0	6.95	9.53
26. *T. viride*	44.88 ^{bc}	19.49	31.76 ^{c}	11.36	50.59 ^{b}	2.12	42.41	14.08
27. *T. piluliferum*	-3.94 ^{jklm}	13.31	3.53 ^{ef}	3.11	0.00 ^{e}	0	-0.14	7.56
28. *T. viride*	27.17 ^{cdef}	1.8	8.43 ^{def}	4.42	0.00 ^{e}	0	11.87	12.28
29. *T. viride*	14.17 ^{efghi}	12.12	0.00 ^{f}	0	0.00 ^{e}	0	4.72	9.32
30. *T. virens*	15.35 ^{efgh}	9.55	15.29 ^{cdef}	4.08	11.76 ^{d}	0	14.14	5.49
CD at 5%	17.88		19.08		9.13			

Table 2: Sclerotia formed under different *Trichoderma* spp. treatments and their germination on PDA*

Isolate	No. of sclerotia Average	+ SD	Transformed Average	+ SD	Germination (%)
1. *Trichoderma harzianum*	76	5.13	8.76^{k}	0.29	100
2. *T. harzianum*	104	10.97	10.19^{hijk}	0.53	100
3. *T. viride*	109	7.94	10.45^{fghijk}	0.38	98.33
4. *T. viride*	121	4.16	11.03^{fghi}	0.19	96.67
5. *T. viride*	106	10.07	10.32^{ghijk}	0.49	93.33
6. *T. viride*	105	9.87	10.24^{ghijk}	0.48	95
7. *T. hamatum*	149	34.78	12.16^{defg}	1.39	100
8. *T. viride*	106	16.5	10.28^{ghijk}	0.79	85
9. *T. viride*	89	2.65	9.45^{ijk}	0.14	86.67
10. *T. harzianum*	1	0.58	1.30^{q}	0.21	0
11. *T. viride*	166	12.17	12.89^{cde}	0.47	85
12. *T. hamatum*	142	30.12	11.89^{defgh}	1.25	100
13. *T. viride*	28	4.36	5.32^{lmn}	0.4	65
14. *T. reesei*	152	28.68	12.29^{def}	1.16	88.33
15. *T. viride*	207	20.43	14.38^{abc}	0.7	100
16. *T. hamatum*	117	6.11	10.82^{fghij}	0.28	88.33
17. *T. harzianum*	130	12.66	11.39^{efghi}	0.55	86.67
18. *T. harzianum*	21	4.16	4.65^{mn}	0.44	1.67
19. *T. reesei*	178	10.41	13.36^{dcd}	0.39	100
20. *T. harzianum*	14	1.73	3.79^{nop}	0.23	0

Table 2 : Cont.....

Isolate	No. of sclerotia Average	+ SD	Transformed Average	+ SD	Germination (%)
21. *T. harzianum*	6	1.73	2.51^{opq}	0.33	0
22. *T. harzianum*	38	21.78	6.00^{lm}	1.73	46.67
23. *T. harzianum*	5	1	2.31^{pq}	0.22	0
24. *T. reesei*	114	10.02	10.67^{fghijk}	0.46	88.33
25. *T. harzianum*	80	17.62	8.95^{jk}	0.96	56.67
26. *T. viride*	46	17.78	6.73^{l}	1.27	78.33
27. *T. piluliferum*	251	31.9	15.84^{a}	0.99	100
28. *T. viride*	224	13.11	14.97^{a}	0.44	98.33
29. *T. viride*	101	2.65	10.07^{fghij}	0.13	85
30. *T. virens*	17	4.73	4.10^{no}	0.56	0
Control	111	85.42	10.06^{hijk}	3.92	100
CD at 5%			1.63		

*Sclerotia harvested from 12 days after incubation from dual culture treated plates

species of *Trichoderma* was found antagonistic to *sclerotium rolfsii* in volatile metabolites. Most antagonist not restrict the growth of pathogen but none of antagonist was able to restrict the growth completely Fig.1 and 2. The comparison of growth inhibition by volatile metabolites of antagonists revealed that maximum efficiency was shown by only T18 isolate of *T. harzianum* was found to provide 89.02% inhibition of the pathogen. Isolate T20 of *T. harzianum* showed 35.29% inhibition of the pathogen; rest of the *T. harzianum* had no effect. In case of *T. viride*, the volatiles from isolates T11 and

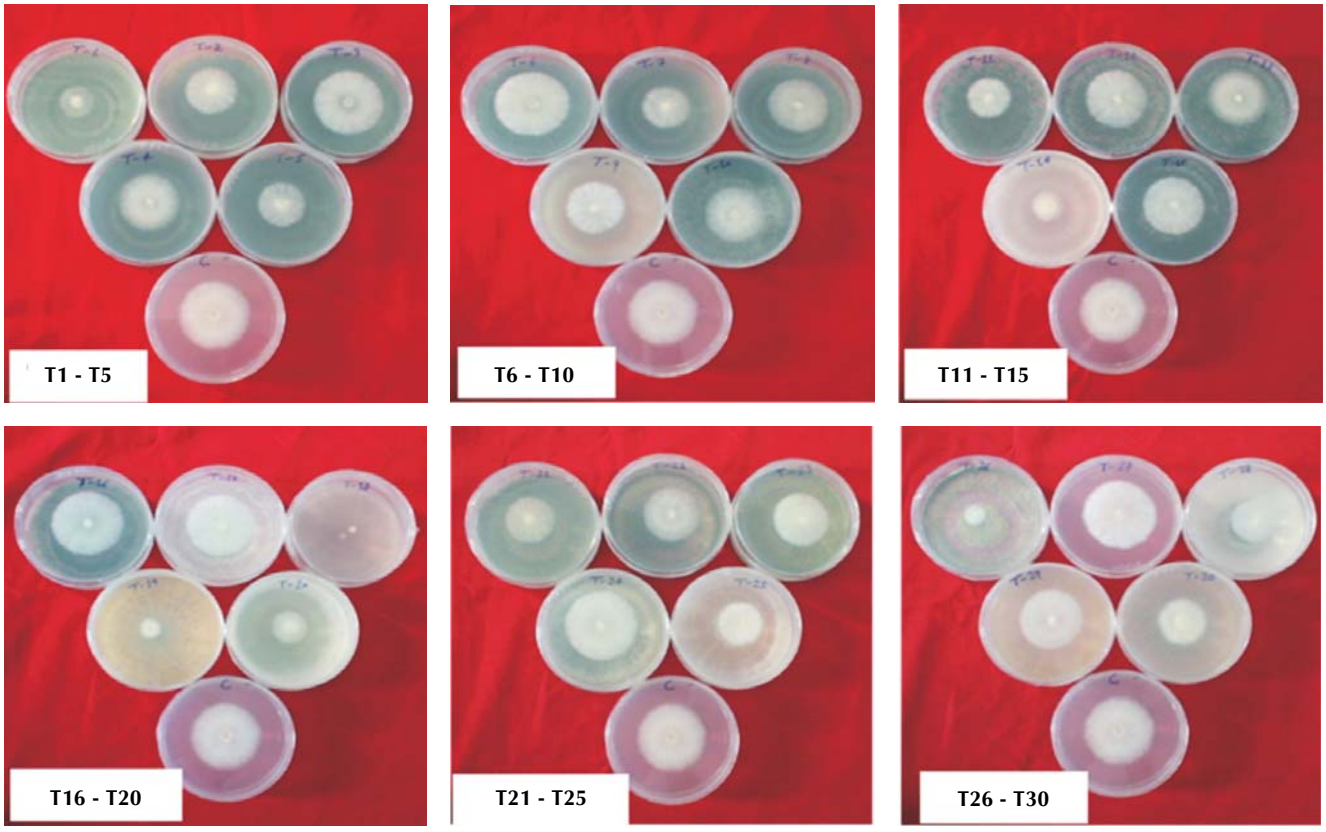

Figure 1: Effect of volatile metabolites of different antagonists against *Sclerotium rolfsii* after 2 days of inoculation with the pathogen

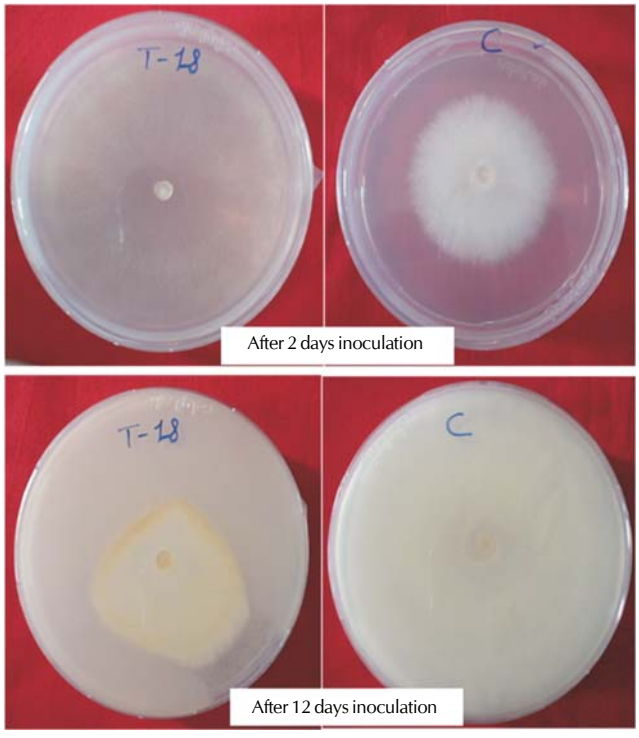

Figure 2: Effect of volatiles of isolate T18 against *S. rolfsii* after 2 days (zero growth of pathogen) and 12 days of inoculation (mycelium degraded due to volatile effect of antagonist of isolate T18

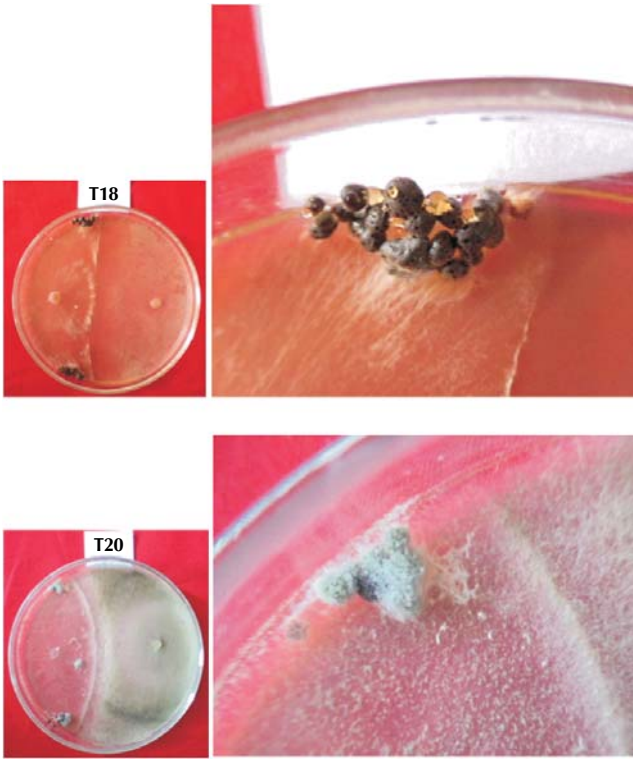

Figure 3: Degeneration of the formed sclerotia in *T. harzianum* isolate T18 and sclerotial parasitization in *T. viride* isolate T20

T26 restricted the growth by 17.65 and 50.59%. Isolate *T. virens* (T30) was found to provide only 11.76% inhibition of the pathogen. All other *Trichoderma* spp isolates had no perceptible effect on the pathogen's growth (Table 1; Fig.1). At the end of 12 days of inoculation with the pathogen, mycelial degradation was evident in case of *T. harzianum* T18 isolate (Fig.2). With respect to *T. virens*, out of the 12 isolates tested, only T26 was effective agaist the against *S. rolfsii* . None of other three species of *Trichoderma* (*T. hamatum*, *T. piluliferum* or *T. reesei*) other than *T. harzianum* or *T. viride*. *T. virens* was unique as it had high level of specificity as it was effective only against *S. Rolfsii*. The inhibitory effects observed here were mainly attributed to the antibiosis by volatile metabolites and culture filtrates of *Trichoderma* isolates simmilar results were made by Mukhopadhyay (1996) in addition to presence of some lytic enzymes in culture filtrates (Srivastava and Singh, 2000). *Trichoderma* produces several volatile compound such as Ethylene, Hydrogen cyanine, Aldehydes and Ketones which play an important role in controlling the plant pathogens Vey *et al*. (2001). The volatile and non volatile compound from *Trichoderma* effectively inhibited the growth of *Colletotrichum capsici* Ajith P.S. and N. Lakshmidevi (2010). Muthukumar *et al*.(2011) and Christy Jeyaseelan *et al*.(2012) recorded maximum growth inhibition of *T .viride* against *Pythium aphanidermatum* through more production of volatile and non volatile compounds. Kumar *et al*. (2012) also reported that the *Trichoderma harzianum* was potential antagonistic activity against *S. rolfsii*.

Sclerotia formation under different isolates of *Trichoderma* spp. and their germination

Number of sclerotia formed under different treatments of *Trichoderma* spp. and their germination on PDA is presented in (Table 3). Of the 14 *Trichoderma* spp. isolates of *viz*., T1, T2, T3, T4, T5, T6, T8, T9, T12, T16, T17, T24, T25 and T29 there were no differences in the number of sclerotia formed as compared to control. In other 8 isolates (T7, T11, T14, T15, T19, T27, T28 and T29) number of sclerotia was significantly higher as compared to control. In another 8 isolates (T10, T13, T18, T20, T21, T22, T23, T26 and T30) the number of sclerotia formed was significantly less than control.

It was interesting to note that the significant volatile inhibitory activity of T18 isolate recorded against *S. rolfsii* also reflected in significantly less sclerotial formation. A cross section of sclerotia formation in some treatments is presented in Fig. 3 where inhibition (T18 and T20) and excessive sclerotia formation (T19 and T15) compared to the control. A close-up view Fig.3 shows how an isolate (T18) aborted the sclerotia formed or colonized and destroyed the sclerotia (T20). The germination test results (Table 2) also indicate that though sclerotia formed in some of the isolates of *Trichoderma*, these were ultimately destructed resulting in their poor germination. Sarma and Singh (2003) have reported that the ferulic acids are the major inhibitory factor of *S. rolfsii*. Several reports indicated the atagonistic mechanisms of Trichoderma demonstrated the involvement of many hydrolytic enzymes (Sanz *et al*., 2004), also capable of acting synergistically with highly fungitoxic antibiotics and a complex system for fungal prey detection (Lorito *et al*., 2010). These results suggest that bioagents strain T18 native isolates of (*Trichoderma harzianum*) produce a range of volatile metabolites having varying degree of effects on sclerotial test fungi. Such studies are important for selection of effective antagonistic for the management of soil borne diseases.

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