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Efficacy of Trichoderma against *Sclerotium rolfsii* causing collar rot disease of lentil under *in vitro* conditions

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Abstract: Three biocontrol agents *viz., Trichoderma viride*, *T. virens* and *T. harzianum* were evaluated to test the antagonism against *Sclerotium rolfsii* under *in vitro* conditions. All the three antagonists' viz., *T. viride, T. virens* and *T. harzianum* have shown the potential of parasitizing the growth of *Sclerotium rolfsii in vitro.* The rate of inhibition was fastest in *T. harzianum* (63.60%) followed by *T virens* (51.5 %). Least inhibition was recorded in *T. viride (50.85%)* after 72 hours of incubation. However, *T. viride* showed the highest (91.31%) reduction in sclerotia formation followed by *T. harzianum* (84.92%) and *T. virens* (84.29%) after 15 days of incubation. The volatile compounds from *Trichoderma viride* were found most effective in suppressing the mycelial growth (51.11%) and sclerotia production (95.90%) of the target pathogen. The culture filtrate from both *T. harzianum* and *T. viride* (15% concentration) was found very effective in inhibiting the radial growth (57.46 and 49.62%) and sclerotia formation (98.20 and 99.83%) of *Sclerotium rolfsii.* The antagonists such as *T. harzianum* and *T. viride* can be used as a bio-control agent against *S. rolfsii* under field condition.

Keywords: Collar rot, Efficacy, Lentil, *Sclerotium rolfsii*, Trichoderma

INTRODUCTION

Lentil production in India has always been important as it is one of the most important Rabi crops in the country. Lentil has potential to grow in dry land areas. It is also used as a cover crop to check the soil erosion and is grown throughout the northern and central India. In India, it occupies an area of 1.48 m ha and contributes 1.03 m t to pulse production with the productivity of 697 kg ha-1. It is mainly cultivated in Madhya Pradesh, Uttar Pradesh, Bihar, and West Bengal, which account for 85 % of total production (Anonymous, 2015). The crop is both cultivated as a primary crop as well as a secondary crop in the districts like Sagar, Jabalpur, Bundelkhand and Bhopal in Madhya Pradesh. However, in Madhya Pradesh, lentil yield potential is far below (711.93 kg/ha) than the other cereal crops (Mondal *et al*., 2013). Various causes are associated with its low yield. One of them is the diseases causing remarkable yield loss. Lentil suffers from an attack of a number seed borne diseases such as vascular wilt, collar rot, root rot, stem rot, rust, powdery mildew and downy mildew, which are caused by *Fusarium oxysporum* f.sp. *lentis, Sclerotium rolfsii, Rhizoctonia solani, Uromycis fabae, Erysiphe polygoni and Peronospora lentis,* respectively (Khalequzzaman, 2016). Among the diseases, collar rot caused by *S. rolfsii* which is gaining importance. *S. rolfsii* is an economically important pathogen on numerous crops worldwide. It has an extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers and cucurbits, and commonly occurs in the tropics, subtropics, and other warm temperate regions (Hemanth *et al*., 2016). Management of soil borne plant pathogens including *S. rolfsii* can be achieved by different fungicides, soil fumigants (Methyl bromide) and bioagents. Frequent application of fungicides causes environmental pollution therefore there is a need to reduce the amount of chemicals applied to soil (Hemanth *et al*., 2016). The fast growth of the *S. rolfsii* and its capability of producing excessive sclerotia that may persist in soil for several years (Singh *et al.,* 2012). Hence management of *S. rolfsii* causing collar rot of lentil is difficult to achieve chemically, In this context bioagents can be used as an alternative source for controlling soil-borne diseases since they comprise a rich source of bioactive substance ((Jegathambigai *et al.,* 2010). Biological control of plant diseases has been the subject of extensive research in the last two decades. Trichoderma spp. is well documented as effective biological control agents of plant diseases (Sain and Pandey, 2016). Therefore, the present investigation was carried out to evaluate the *Trichoderma* against *S. rolfsii* causing collar rot of lentil.

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MATERIALS AND METHODS

The experiments were conducted in the Department of Plant Pathology J.N.K.V.V. Jabalpur (M.P.) during 2015-16. Three biocontrol agents *viz., Trichoderma viride*, *T. virens* and *T. harzianum* were evaluated to test the antagonism against *S. rolfsii* causing collar rot of lentil.

Growth of antagonist and the pathogen in monoculture and dual culture: To study the growth of antagonists and the test fungus in monoculture, 5 mm mycelial discs of *T. viride*, *T. virens, T. harzianum* and *S. rolfsii* were inoculated centrally on sterilized potato dextrose agar in Petri-dishes. Then plates were incubated in BOD incubator at 28 $\pm$ 1^0C. Observations on colony diameter of individual antagonist and the pathogen were recorded after 72 hrs of incubation. For screening of the antagonists against *S. rolfsii,* dual culture technique developed by Mortan and Straube, (1955) was adopted. Observation on colony diameter of bioagents and test fungus was recorded. Inhibition of mycelial growth of test pathogen over check was calculated by the formula given by Vincent (1947).

I (%) = (C-T)/C*100

Where, I = percent inhibition, C= colony diameter in control, and T= Colony diameter in treatment

Re-isolation was done by transferring 5 mm mycelial disc cut by cork borer from the zone where the test fungus was already overgrown by the antagonist on PDA medium to study the viability of test fungus

Effect of volatile and non volatile compounds from antagonist(s) on the radial growth of *S. rolfsii:* The effect of volatile compounds from *T. viride, T. virens and T. harzianum* on radial growth of *S. rolfsii* was followed as per the method given by Dennis and Webster (1971a and b). The two bottom portion of petriplates containing PDA were inoculated with a 5 mm disc of pathogen and antagonist, respectively and both inoculated bottom plates were placed facing each other and sealed with cellophane adhesive tape. The petriplate containing PDA without antagonist serves as control. The observations on the radial growth of the test fungus were recorded after 3 days and formation of sclerotia after 15 days of incubation at 28 ± 1ºC.

To study the effect of non volatile compounds, the bio-control agents were grown in Potato dextrose broth at 27ºC with intermittent shaking at 150 rpm. The metabolites were collected after 15 days and filtered. The sterilized filtrate was amended in PDA to make 5, 10 and 15% concentration in petriplates. The solidified agar plates in triplicates were inoculated at the centre with 5 mm diameter mycelial disc of the pathogen and incubated at 28ºC for 3 days. The Plates without filtrate served as control. The Colony diameter and sclerotia formation were measured (Chaurasia *et al.,* 2013) and percent inhibition of radial growth and sclerotia formation was calculated using the formula given by Vincent 1947.

RESULTS

In monoculture, *T. viride* showed 90 mm growth on PDA after 72 hrs of incubation followed by *T. virens* and *T. harzianum* which exhibited 87.33 mm and 86.50 mm colony diameter, respectively. *S. rolfsii* showed 89.33 mm growth on PDA after 72 hrs of incubation. In dual culture, all the three antagonists' viz.,

Table 1. Effect of Trichoderma on growth and sclerotia formation of *S. rolfsii.*

	Monoculture	Dual culture				
Treatment	**Colony diameter (mm)***	**Colony diameter of antagonist (mm)***	**Colony diame-ter of Pathogen (mm)***	**Growth Inhibition (%)**	**No. of sclerotia formed**	**Sclerotia Inhibiton over contol**
Trichoderma viride	90.00	45.76	44.23	50.85	36.66	91.31
Trichoderma virens	87.33	46.83	43.16	51.55	66.33	84.29
Trichoderma harzianum	86.50	56.46	33.53	63.60	63.66	84.92
Sclerotium rolfsii	89.33		36.86	-	422.33	
CD (0.05)	2.358		2.556		3.619	

*Average of 3 replication

Table 2. Effect of volatile and non-volatile compounds from *Trichoderma* on radial growth of *Sclerotium rolfsii* after three days of incubation.

	Volatile compounds		Non - volatile compounds					
			5%		10%		15%	
Treatment	**Radial growth of mycelium (mm)***	**Growth inhibition (%)**	**Myceli-algrowth of pathogen (mm)***	**Growth Inhibi-tion (%)**	**Myceli-algrowth of pathogen (mm)***	**Growth Inhibi-tion (%)**	**Myceli-algrowth of pathogen (mm)***	**Growth Inhibi-tion (%)**
Trichoderma harzianum	54.00	40.00	87.33	02.33	77.50	13.24	38.00	57.46
Trichoderma viride	44.00	51.11	89.00	00.36	82.50	07.64	45.00	49.62
Trichoderma Virens	45.00	50.00	89.00	00.36	83.16	06.90	65.66	26.49
Sclerotium rolfsii	90.00	--	89.33	--	89.33	--	89.33	--
CD (0.05)	2.325		N\A		3.195		3.436	

Table 3. Effect of volatile and non-volatile compounds from *Trichoderma* on Sclerotia formation of *Sclerotium rolfsii* after fifteen days of incubation.

Treatment	Volatile compounds		Non - volatile compounds					
			5%		10%		15%	
	No. of sclero-tia formed (15 DAI)*	Sclerotia inhibition (%)	No. of scle-rotia formed (15 DAI)*	Sclerotia inhibition (%)	No. of sclerotia formed (15 DAI)*	Sclerotia inhibi-tion (%)	No. of sclerotia formed (15 DAI)*	Sclerotia inhibition (%)
Trichoderma harzianum	218.00	49.49	598.66	02.44	196.33	68.00	11.00	98.20
Trichoderma viride	17.66	95.90	602.66	01.79	319.66	47.90	01.00	99.83
Trichoderma Virens	151.66	64.86	581.66	05.21	302.66	50.67	113.33	81.53
Sclerotium rolfsii	431.66	--	613.66	--	613.66	--	613.66	--
CD (0.05)	4.167		3.744		2.921		2.972	

*Average of 3 replications

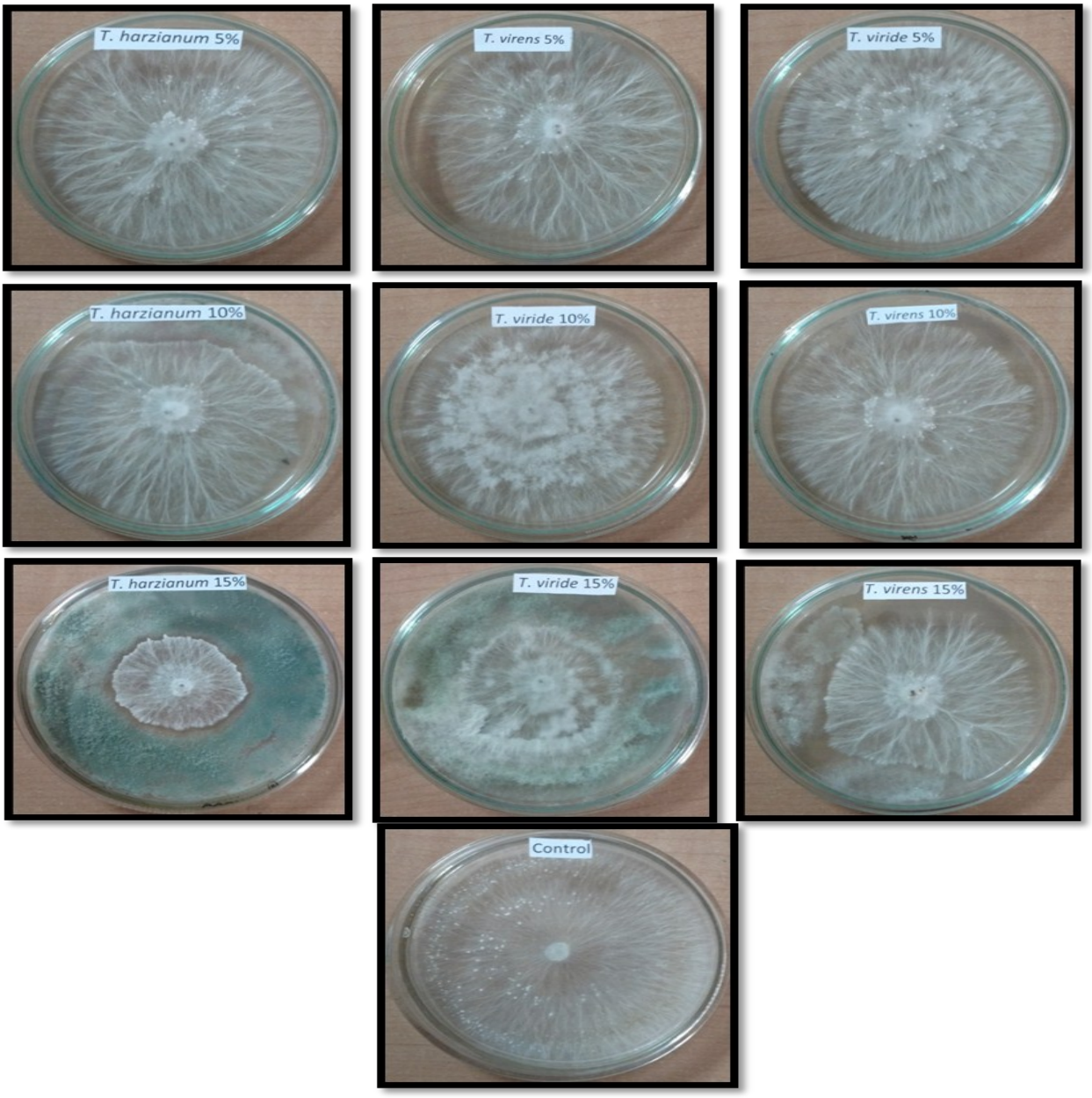

Plate 1. *Effect of non - volatile compounds from Trichoderma on radial growth of Sclerotium rolfsii.*

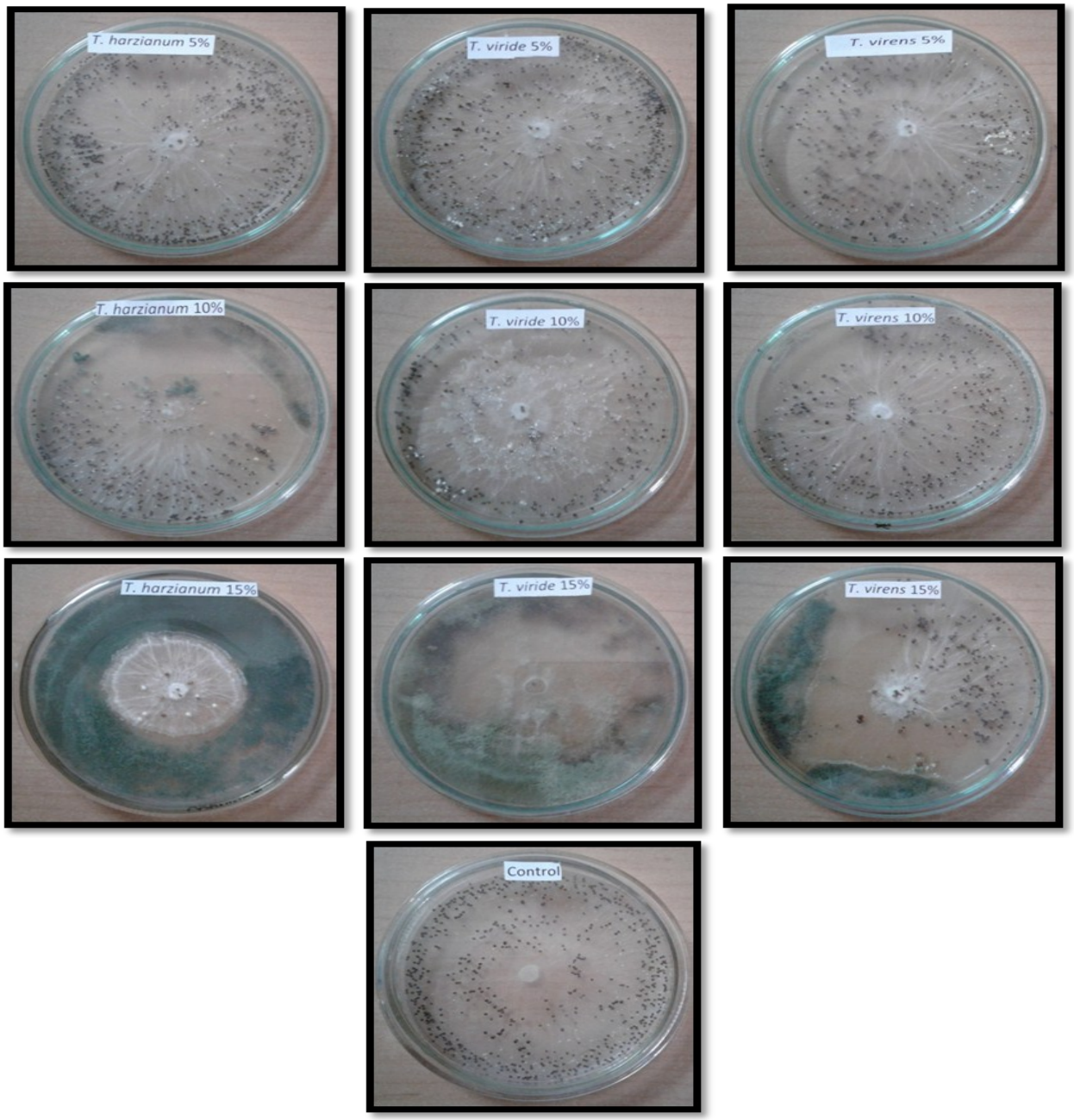

Plate 2. *Effect of non - volatile compounds from Trichoderma on sclerotia formation of Sclerotium rolfsii.*

T. viride, T. virens and *T. harzianum* have shown the potential of parasitizing the growth of *Sclerotium rolfsii in vitro*. The rate of inhibition was fastest in *T. harzianum* (63.60%) followed by *T. virens* (51.5 %) and *T. viride* (50.85%) after 72 hours of incubation. However, *T. viride* showed the highest (91.31%) reduction in sclerotia formation followed by *T. harzianum* (84.92%) and *T. virens* (84.29%) after 15 days of incubation (Table-1).

The effect of volatile compounds from Trichoderma species on the growth and sclerotia formation of *Sclerotium rolfsii* revealed that all the *Trichoderma* species produced toxic volatile metabolites having significant effect in reducing the radial growth and sclerotia formation of the test pathogen. The volatile metabolites from *Trichoderma viride* were most effective in inhibiting the mycelial growth (51.11%) and sclerotia production (95.90 %) followed by *T. virens* (50% and 64.86%). The least inhibition of mycelia growth (40 %) and sclerotia production (54%) was recorded in *T. harzianum* (Table-2). The non–volatile compounds from all the three Trichoderma species at 5 and 10% concentration were found not effective in inhibiting the radial growth and sclerotia formation of *S. rolfsii* however, were found very effective at 15% percent concentration. The non volatile metabolites from *T. harzi-*

anum at 15% percent concentration was most effective in inhibiting the mycelial growth (57. 46%) of target pathogen followed by *T. viride* (49.62%) . The least inhibition of mycelial growth (26.49%) was recorded in *T. virens.* However, the non volatile metabolites from *T. viride* at 15% percent concentration were most effective in inhibiting the sclerotia production (99.83%) of target pathogen followed by *T. harzianum* (98.20%). But they were significantly at par with each other. The least inhibition of sclerotia production (81.53 %) was recorded in *T. virens (*Table-2, Plates-1 and 2).

DISCUSSION

Antagonisim of Trichoderma species against several pathogens were reported by Reddy *et al*. (2013), Sundara moorthy and Balabaskar (2013), Hanan and Mohamed (2014). The degree of inhibition varied from one strain to another. Species of Trichoderma have been demonstrated *in vitro* to act against fungal plant pathogens by producing diffusible volatile antibiotics. Vey *et al*. (2001) reported that there are large varieties of volatile secondary metabolites produced by Trichoderma such as ethylene, hydrogen cyanide, aldehydes and ketones which play an important role in controlling the plant pathogens (Bhagat *et al*., 2014). Similarly, Amin *et al*., (2010) reported volatile activity of six isolates of Trichoderma spp. against seven different fungal plant pathogens. Among the six Trichoderma isolates studied, *T. viride* (Tv-1) was found to most effective in reducing the mycelial growth of *F. oxysporum* (41.8%). *S. rolfsii* mycelium growth and sclerotial production were inhibited by 40.68 and 48.1 %, respectively. In case of *R. solani*, *T. viride* (Tv-2) accounted for maximum reduction in mycelial growth (30.58%) and sclerotial parasitization (65.6%). Several workers like Pan and Bhagat (2008), Stoppacher *et al*. (2010) and Pan *et al*. (2013) have also reported the effectiveness of diffusible volatile compounds by *T. viride* and *T. harzianum* under *in vitro*. Antifungal volatile compounds produced by strain SQR-T037 highly effective to suppress the growth of *F. oxysporum* up to 9 days causing watermelon wilt (Waseem *et al*., 2013) In the present investigation, *T. viride* and *T. harzianum* were highly efficient whereas *T . virens* have exhibited relatively less inhibition of mycelial growth of test fungus. The possible reason may be due to their inherent potentiality to adapt well in introduced conditions and aggressiveness of the Trichoderma isolates towards certain plant pathogens (Pan and Jash, 2009). Several workers studied on the production of volatile and non-volatile antibiotics revealed that *T. harzianum* and *T. viride* were highly effective in reducing the radial growth of *S. rolfsii* by the production of these substances (Rao and Kulkarni, 2003). Dubey and Suresh (2006) found that non-volatile substances produced by *T. harzianum* are inhibitory to *F. o. f. sp. ciceri* causing chickpea wilt. Similarly, *T. viride* isolate, followed by *T. harzianum* inhibited maximum mycelial growth of the *F. o. f. sp. ciceri* through production of volatile and non-volatile inhibitors in dual culture (Dubey *et al*., 2007). Waseem *et al*., (2013) reported that nonvolatile antifungal compounds extracted from the liquid culture Trichoderma strain SQRT037, significantly inhibited the growth of *F. oxysporum. f.sp. niveum* incitant watermelon of wilt. Nagamani *et al*. (2017) reported that Trichoderma, spp. are the most promising and effective bioagents against many plant pathogenic fungi. They screened twenty Trichoderma isolates for their efficacy against soil borne plant pathogens namely *R. bataticola, F. oxysporum ciceri* and *S. rolfsii* in chickpea. The isolates *T. asperellum* (ATPU 1), *T. harzianum* (ATPP 6), *T. asperelum* (KNO 2), *T. asperellum* (KNPG 3) were most efficient in the production of volatile and non-volatile compounds.

Conclusion

From the *in vitro* findings, it can be suggested that the antagonists such as *Trichoderma harzianum* and *Trichoderma viride* can be used as a bio-control agent against *Sclerotium rolfsii* under field condition. It is also revealed that the microorganisms that naturally remain in the soil are having more or less similar potential antagonistic effect on the various crop disease caused by various pathogens. And some of them can be used as a potential bio- control agent under field condition to decrease the disease incidence and to increase crop productivity. Therefore, further work should be taken up to explore the possibility of the use of the antagonists under study in field condition for the biological control of the diseases caused by *Sclerotium rolfsii*.

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