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BIOCONTROL POTENTIALS OF *TRICHODERMA HARZIANUM* AGAINST SCLEROTIAL FUNGI

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ABSTRACT
Trichoderma species are well known antagonists which have strong bio-control potential against soilborne phytopathogenic fungi. Five potential isolates of *Trichoderma hazianum* were isolated from two different types of soils in which two were from alfisol (mango and litchi orchards) of the ICAR RCER Research Centre, Plandu, Ranchi, Jharkhand, India and three were from the inceptisol (Eastern Uttar Pradesh) India using *Trichoderma* Selective Medium (TSM) and characterized. Two alfisol (native) isolates (Th-4 and Th-5) along with three inceptisol isolates of Varanasi (Th-1, Th-2 and Th-3) were used in screening of antagonistic potential against two phyto-pathogenic sclerotial fungi *Rhizoctonia solani* and *Sclerotium rolfsii* in dual culture. Among the isolates, the local/native ones isolated from alfisol have shown potential antagonism and inhibited *R. solani* with percent inhibition of 46.77 and 50.34 as compared to inceptisol isolates with inhibition percentage of 22.33, 26.21 and 23.08 respectively. The alfisol isolates also showed potential antagonism against *S. rolfsii* with percent inhibition of 44.67 and 47.88 as compared to inceptisol isolates with inhibition percentage of 3.97, 7.97 and 28.72 respectively. Compared to inceptisol isolates, biomass accumulation and total phenol content was also reported high in the alfisol isolates.

INTRODUCTION

Trichoderma are free-living soilborne fungi which are highly interactive in the rhizosphere and foliar environments. *Trichoderma* are known as imperfect fungi but now their perfect stage (*Hypocrea*) is known, are fast growing in culture and produce numerous green spores and chlamydospores. *Trichoderma* have created ecofriendly, safe and non-chemical disease management system which have great importance in organic agriculture. *Trichoderma,* a soilborne mycoparasitic fungus has been shown effective against many soil borne phytopathogens (Papavizas, 1985; Herman *et al.,* 1998; Herman, 2000; Pan *et al.,* 2001; Jash *et al.,* 2004; Herman, 2006; Maurya *et al*., 2008, Rajkonda *et al.* 2011, Dolatabadi *et al*. 2012). Biological control of soil borne phytopathogens has been the subject of extensive research in the last few decades. However, with the increasing interest in biological control, owing to environmental and economic concerns, thousands of research experiments are going on for searching novel, potential, safe and have ability to inhibit wide range of soilborne phytopathogens. *Trichoderma* spp. is well documented as effective biological control agents of soilborne 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* Rafai 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; Elad *et al.*, 1980; Henis *et al.,* 1983; Pande, 1985; Patil, 1993; Kamala *et al.,* 2012). Due to the knowledge of their potentials, several *Trichoderma* based several commercial products are manufactured and marketed in Asia, Europe and USA worldwide for the management of plant diseases (Harman *et al.*, 2004; Herman *et al.*, 2006).

Biological control of soilborne plant pathogens can be achieved successfully by seed coating, furrow application and root dip of seedlings with Antagonists. Harman *et al.* (1980) reported the biocontrol of *S. rolfsii* and *Pythium* spp. by coating radish and pea seed with *T. hamatum*. Hadar *et al*. (1979) and 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. Many researchers have demonstrated that the potential of *Trichoderma* sp. in controlling wilt and damping-off diseases of crop plants caused by *Fusarium* sp. and *Rhizoctonia solani* (Rojo *et al.,* 2007; Kamala *et al.,* 2012).

Sclerotium rolfsii Sacc., [teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough] and *Rhizoctonia solani*. Kuhn. (teleomorph = *Thanatephorus cucumeris* Frank (Donk)) are important soil-borne phytopathogens which are very common in tropical, subtropical and temperate regions of the world. Both the phytopathogens are survives in the form of vegetative mycelium and/or sclerotia and causes several diseases in crop plants and infected more than 500 species of cultivated and wild plants (Aycock, 1966; Punja, 1985; Ciancio and Mukerji, 2007; Maurya, 2007; Maurya *et al*., 2008; Yaqub *et al*., 2011).

Keeping these views in mind, the experiments were designed to find out the potential isolates of *Trichoderma harzianum* for the management of two potent sclerotial fungi viz., *R. solani* and *S. rolfsii* in alfisols of Eastern Plateau and Hill region.

MATERIALS AND METHODS

Collection of soil samples

The present investigation was conducted in plant pathology Laboratory, ICAR, RCER, Research Centre, Plandu, Ranchi, India. Two alfisol soil samples (pH-acidic, N-medium, P-low, K-Sufficient, OC-low) were collected in different localities of ICAR campus and adjoining areas of fruit-tree orchard, mango and litchi rhizosphere, at the depth of 5-7cm of soil surface and three inceptisol soil samples (pH-neutral, N-sufficient, P-normal, K-normal, OC-low to medium) were collected from Varanasi, India. The composite soil samples were collected from a particular field in the polyethylene bag and labelled separately.

Isolation of *Trichoderma* from soil sample

Trichoderma was isolated from the collected soil samples by serial dilution Technique of soil sample. One millilitre of 10^{-4} dilution was poured on to *Trichoderma* selective Medium ($MgSO_4$: 0.20g, KH_2PO_4: 0.90g, NH_4NO_3: 1.0g, KCl: 0.15g, Glucose: 3.0g, PCNB: 20g, Rose Bengal: 0.15g, Chloremphanicol: 0.25g, Agar-agar: 15g, Metalaxyl: 30g, Distilled water: 1L) for isolation of *Trichoderma* and after the appearance of the colonies of *Trichoderma,* purified by hyphal tip isolation techniques. They were identified on the basis of their morphological and microscopic characteristics. The purified and identified cultures of *Trichoderma* spp. were maintained on Potato Dextrose Agar (PDA) medium and stored at 4ºC for further use.

The bioagent- *Trichoderma*

The genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or postulate repetitively branched conidiophore with lageniform phialides and hyaline or green conidia born in slimy heads. The primary branches of conidiophore produce smaller secondary branches that also may produce tertiary branch, and so on. Conidia are hyaline usually green, smooth – walled or roughened. Phialides are ampulliform to lageniform, usually constricted at the base, more or less swollen near the middle, and abruptly near the apex into short sub-cylindric neck.

Isolation of the sclerotial fungi

Diseased plants which have the sclerotia of the pathogens were collected from ICAR-RCER Research Centre, Ranchi, Jharkhand. Then after the collected sclerotia were surface sterilized by dipping in 0.1% HgCl for 5-10 second followed by three subsequent washing with sterilized distilled water and then after they were placed in culture plates containing Potato Dextrose Agar (PDA) Medium and incubated at 25 ± 2ºC. The culture were purified and maintained on PDA slants for further experimentation. They produce profuse sclerotia by accumulation of fungal mycelium. Moreover, the development of sclerotia was due the secretion of oxalic acids. 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 collar region of the plants and cause infection. The pathogen has wide host range which infect more than 500 crop plants

Screening of antagonistic potential of *Trichoderma* with sclerotial fungi

Screening of antagonistic potential of *T. harzianum* with sclerotial fungi was assessed by Dual Culture in Petri dish. The mycelial bits of 5mm diameter of *Trichoderma* strain and sclerotial fungi were placed opposite to each other on Petri plates containing PDA in triplicate with one set of control of *S. rolfsii* and *R. solani* without inoculating the *Trichoderma* isolates. The plates were incubated at 24 ± 2ºC. The data were recorded regularly on the growth of the pathogen and *Trichoderma* isolates after 24h interval. The percent inhibition of mycelia growth over control was calculated by following equation (Vincent, 1927).

I% = C-T/C X100

Where, I = Percent inhibition of mycelium, C = Growth of mycelium in control. T = Growth of mycelium in treatment.

Estimation of total phenol content

The content of total phenol was estimated Streptrophotometry using Folin –Ciocalteau Reagent (Bray and Thorpe, 1954).

RESULTS AND DISCUSSION

During the course of experiment, the biocontrol agent *T. harzianum* isolates were cultured individually to study their growth behaviour and antagonistic potential against sclerotial fungi. Every 24h after inoculation, radial growth was recorded and it was observed that both sclerotial fungi and *T. hazianum* is fast grower and it covered 50% area of Petri plates of 90mm diameter within 48h and it covers full on fourth day, *i.e.*, 90h of inoculation (Table 1 and 2, Fig. 3). In the sclerotial fungi, their mycelium produces white tiny pin head sclerotial initials which develop in sclerotia of light brown to dark brown in colour. All the screened isolates of *T. harzianum* showed diverse antagonistic efficacy in dual culture against both the

Table 1: Growth of *T. harzianum* and *R. solani* in Petri dish

Isolates of *T. harzianum*	Growth observation (in mm) Day 1		Day 2		Day 3		Day 4	
	T	R	T	R	T	R	T	R
Th-1	7.78 ± 0.95	5.44 ± 0.48	17.07 ± 0.44	15.40 ± 0.31	31.94 ± 0.34	25.11 ± 0.22	44.78 ± 0.49	34.22 ± 0.11
Th-2	8.33 ± 1.33	5.88 ± 0.44	20.00 ± 2.50	15.54 ± 0.40	33.67 ± 0.38	22.67 ± 0.70	46.22 ± 0.11	32.51 ± 0.41
Th-3	12.33 ± 1.17	5.10 ± 0.49	26.45 ± 1.75	14.67 ± 0.19	40.78 ± 0.59	23.89 ± 0.49	50.84 ± 0.34	33.89 ± 0.22
Th-4	15.29 ± 0.16	5.95 ± 0.36	32.78 ± 1.56	15.11 ± 0.59	52.78 ± 0.73	20.77 ± 0.33	64.00 ± 0.39	23.45 ± 0.22
Th-5	15.67 ± 0.19	5.96 ± 0.15	33.00 ± 0.19	15.22 ± 0.11	54.89 ± 0.29	19.97 ± 0.52	66.60 ± 0.26	21.88 ± 0.29

T = *Trichoderma*, S = *R. solani*, ± Standard error mean

Table 2: Growth of *T. harzianum* and *S. rolfsii* in Petri dish

Strains of *Trichoderma*	Growth observation (in mm) Day 1		Day 2		Day 3		Day 4	
	T	S	T	S	T	S	T	S
Th-1	4.44 ± 2.23	7.44 ± 1.24	11.88 ± 1.87	17.22 ± 0.56	27.44 ± 4.06	31.66 ± 0.51	38.55 ± 3.21	40.11 ± 0.80
Th-2	12.22 ± 1.60	8.11 ± 0.78	27.55 ± 2.47	17.55 ± 1.56	52.33 ± 0.84	27.33 ± 0.84	55.88 ± 0.62	29.77 ± 1.16
Th-3	7.00 ± 1.20	7.22 ± 1.28	15.99 ± 2.03	17.44 ± 2.06	30.89 ± 1.25	32.00 ± 0.51	42.00 ± 0.70	38.44 ± 0.78
Th-4	13.67 ± 0.67	7.55 ± 0.11	33.44 ± 0.56	16.22 ± 0.44	54.77 ± 3.56	19.99 ± 3.33	60.89 ± 2.11	23.11 ± 3.45
Th-5	10.66 ± 0.00	8.66 ± 0.19	32.44 ± 0.11	16.55 ± 0.29	56.33 ± 0.51	19.66 ± 2.68	61.78 ± 0.40	21.77 ± 1.96

T = *Trichoderma*, S = *S. rolfsii*, ± Standard error mean

sclerotial fungi but their antagonistic potentials varied isolate to isolate. Among all the screened isolates, the isolates from alfisols (Th-4 and Th-5) of *T. harzianum* showed strong antagonistic potentials followed by the inceptisol isolates Th-1, Th-2, and Th-3 (Table 2, Fig. 2). It was found that alfisol isolates have strong antagonistic potential on fifth day of inoculation and overlapped the colonies of *R. solani* and *S. rolfsii*. In *S. rolfsii,* the maximum antagonistic potential was observed in Th-5 isolates (47.88%) and Th-4 (44.67%). Moreover, against *R. solani* maximum inhibition potential (50.34%) was also observed in Th-5 followed by 46.77% (Th-4). Antagonistic potential of the isolates of inceptisol against *S. rolfsii* were observed in similar trends but their percent inhibition were

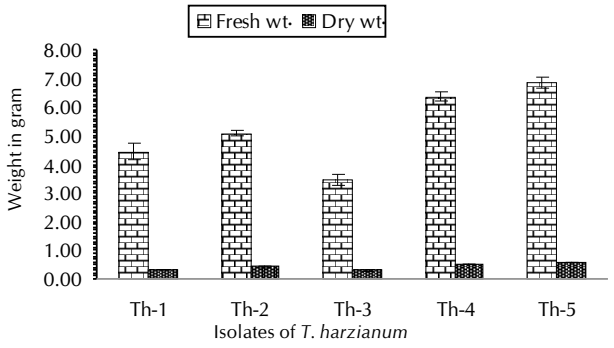

Figure 1: Biomass accumulation by different *T. harzianum* isolates

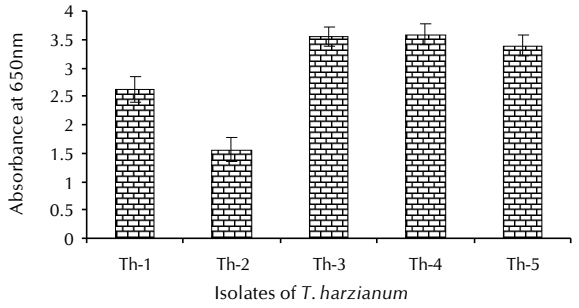

Figure 2: Total phenolic acid in different isolates of *T. harzianum*

Table 3: Percent Inhibition of *R. solani* in dual culture (in mm)

Isolates of *T. harzianum*	Growth of *R. solani* in Treatment	Growth of *R. solani* in Control	Inhibition %age
Th-1	34.22 ± 0.11	44.06 ± 0.52	22.33
Th-2	32.51 ± 0.41	44.06 ± 0.52	26.21
Th-3	33.89 ± 0.22	44.06 ± 0.52	23.08
Th-4	23.45 ± 0.22	44.06 ± 0.52	46.77
Th-5	21.88 ± 0.29	44.06 ± 0.52	50.34

± Standard error mean

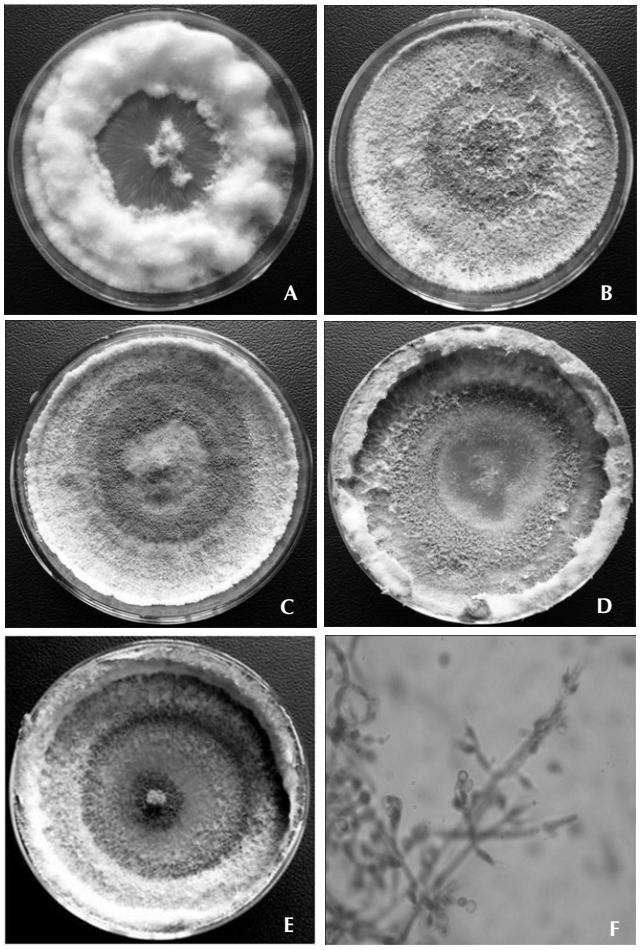

Figure 3: Colony of different *T. harzianum* isolates and on PDA Media (a) Th-1; (b) Th-2; (c) Th-3; (d) Th-4 (e) Th-5; (f) Micrograph of *T. harzianum*

limited as compared to Th-4 and Th-5 (native isolates). When we see the antagonism potential of the isolates of *T. harzianum* which were isolated from the inceptisol were observed maximum (28.73%) in Th-3 followed by 7.97% in Th-2 and 3.97% in Th-1(Table 5, Fig. 5). However, against *R. solani*, the maxi-

Table 4: Percent Inhibition of *S. rolfsii* in dual culture (in mm)

Strains of *Trichoderma*	Growth of *S. rolfsii* in Treatment	Growth of *S. rolfsii* in Control	Inhibition %age
Th-1	40.11 ± 0.80	41.77 ± 1.28	3.97
Th-2	38.44 ± 0.78	41.77 ± 1.28	7.97
Th-3	29.77 ± 1.16	41.77 ± 1.28	28.72
Th-4	23.11 ± 3.45	41.77 ± 1.28	44.67
Th-5	21.77 ± 1.96	41.77 ± 1.28	47.88

± Standard error mean

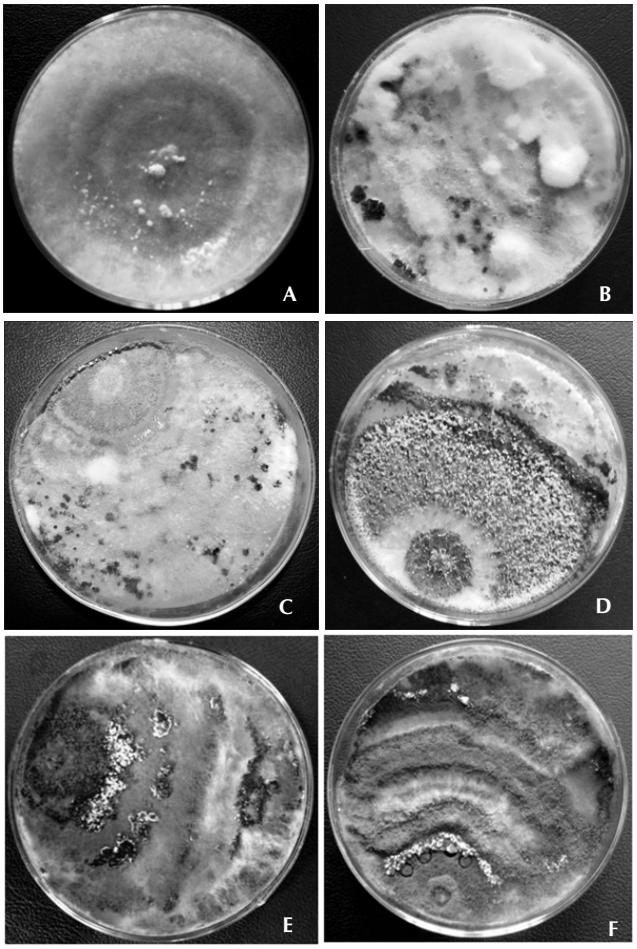

Figure 4: Antagonistic efficacy of different isolates of *T. harzianum* against *R. solani*. (a) *R. solani* (a) Th-1; (b) Th-2; (c) Th-3; (d) Th-4; (e) Th-5

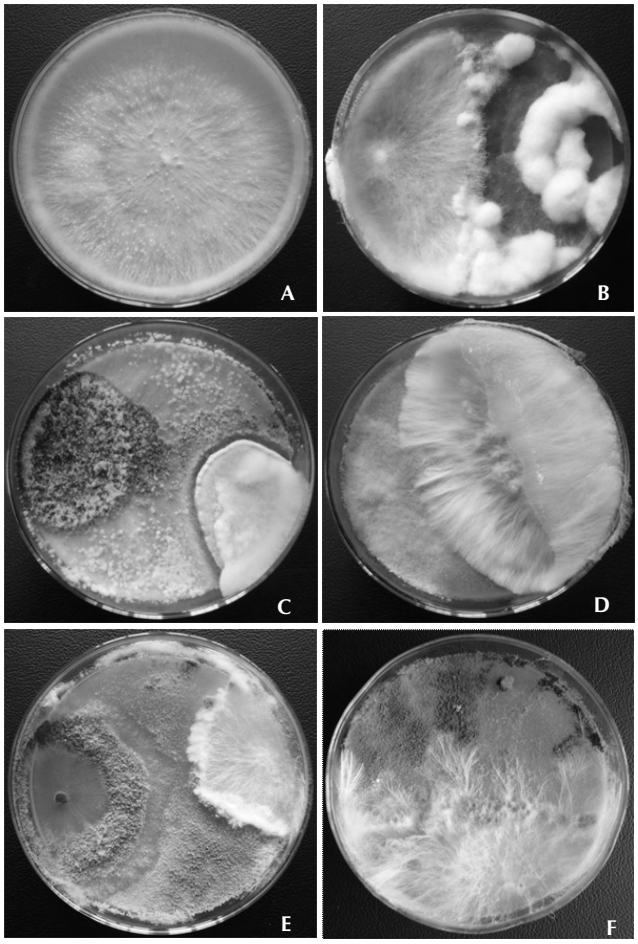

Figure 5: Antagonistic efficacy of different isolates of *T. harzianum* against *S. rolfsii*. (a) *S. rolfsii;* (b) Th-1; (c) Th-2; (d) Th-3; (e) Th-4; (f) Th-5

mum inhibition percent was observed in Th-2 (26.21%) followed by Th-3 (23.08%) and Th-1 (22.33%) (Table 4, Fig. 4). Moreover, when we see the total phenols and biomass in various isolates of *T. harzianum*, Th-4 was showing maximum phenolic content of 3.59mgg^{-1} fresh weight followed by Th-3 (3.58 mgg^{-1}), Th-4 (3.39 mgg^{-1}) but Th-1 and Th-2 were in traces (Fig. 2). However, when we see the biomass accumulation as compared to inceptisol ones with a maximum of 6.83g (fresh wt.) and 0.58g (dry wt.) in Th-5 followed by Th-4 with 6.37g (fresh wt) and 0.51g (dry wt.) (Fig. 1) followed by Th-1, Th-2 and Th-3. The results indicated that the *Trichoderma* species are well known biological control agents which have ability to inhibit wide range of soilborne phytopathogens by direct antagonism or by secreting several cell wall degrading enzymes or by antibiotics (Sivan *et al.,* 1984 and Coley-Smith *et al*., 1991). Sarma and Singh (2003) have reported that the ferulic acids are the major inhibitory factor of *S. rolfsii*. Maurya *et al*., 2007 also reported that the phenolic acids have ability to inhibit the growth and development of *S. rolfsii*. Several reports indicated that the antagonistic 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). It is interesting to report that the biocontrol potential of alfisol isolates was higher due to higher amount of total phenolic acid. This phenomenon is possibly due to the fact that the alfisols isolates have ability to produce rich amount of secondary metabolites is indicators of stress tolerant as compared to inceptisol isolates of *T. harzianum*. Moreover, the fresh and dry weight accumulation was also observed very high in the native isolates which indicate proliferation and competitive saprophytic ability of the isolates. Antagonistic potentials of alfisol isolates of *T. harzianum* may be due to a number of reasons, including strong saprophytic ability, pathogenecity of isolates, micro- and macroclimatic adaptability, influence of the pathogen origin. Therefore, Th-5 and Th-4 isolate native isolates of *T. harzianum* of Eastern Plateau Hill region (alfisol) could be an excellent candidate for providing long-term biological disease control solution against sclerotial soilborne phytopathogens viz., *R. solani* and *S. rolfsii* and may be exploited in reducing the pesticides load.

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