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ANTAGONISTIC EFFECT OF *Trichoderma* ISOLATES ON *Sclerotium rolfsii*

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ABSTRACT

Present study was carried out with an aim to screen potential *Trichoderma* strains to control *Sclerotium rolfsii*. Among the eleven isolates, Seven (T₁, T₃, T₄, T₅, T₇, T₉ and T₁₁) were preliminarily identified as *T. harzianum* while the rest four (T₂, T₆, T₈ and T₁₀) were identified as *T. viride*. Further, results of study suggested that isolate T₃, T₄, T₁₁, T₂ and T₁₀ were effective against *S. rolfsii*. Later, internal transcribed spacer (ITS)-1 sequence analysis confirmed isolate T₃ as *T. harzianum* and T₁₀ as *T. viride*. In dual culture plate technique, *T. harzianum* isolate T₃ gave highest inhibition of 71.67%, while *T. viride* isolate T₁₀ stood second with an inhibition of 67.23%. *Trichoderma* isolate T₁₀ culture filtrate was found to be the most effective in non-volatile antimicrobial compound production. At 15% (v/v) concentration level, it can totally suppress mycelium growth of *S. rolfsii*. Further, *T. viride* isolate T₂ showed maximum potential in volatile antagonistic compound production with 53.26% radial growth inhibition, followed it with isolate T₃ (52.17% of growth inhibition). All the five antagonistic isolates suppressed sclerotial germination and completely killed sclerotia within 20 days; only isolate T₁₁ required 25 days. Isolate T₁₀ was the most effective in germination suppression.

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1 Introduction

Sclerotium rolfsii, a phytopathogenic fungus, often plays spoil sport in way of increasing betelvine production. It is a familiar soil borne polyphagous fungus, mainly dispersed in tropical and subtropical regions where soil temperature settles around 30°C (Harlapur, 1988). This fungus does not survive for a long period in most of the northern European countries as these regions are in fact excessively cold for this organism to survive. Practical, safe and fully satisfactory usable methods of *S. rolfsii* control have yet to be established. Use of chemical fungicides is limited due to high cost, wide host range and long persistence of sclerotia in soil (Pratt & Rowe, 2002). However, difficulties in chemical mediated fungal control can also arise from regional climate change, position of land, atmospheric condition of field etc. Realizing environmental concern regarding over use of hazardous chemical fungicides to excel crop growth, greater emphasis is given on biological control to reduce environmental hazards, antifungal resistant strain development and cost of cultivation. Biological control of soil borne pathogens can offer environmentally safe, durable and cost effective alternative to chemicals (Erkol et al., 2011; Shafique et al., 2016). Biological control of plant diseases has been the subject of extensive research in last two decades. *Trichoderma* spp. have been reported and accomplished in controlling fungal phytopathogens in various cases (Kubicek, 2001; Harman et al., 2004; Celar & Valic, 2005; Schwarze et al., 2012; Lee et al., 2012; Li et al., 2016). Although a lot of investigators have reported biocidal efficacy of *Trichoderma* spp. against number of soil borne plant pathogens, there is slight information obtainable on use of *Trichoderma* spp. as biocontrol agent against *S. rolfsii*, stem rot causing agent in betelvine. In view of the above research findings, present study was carried out with an aim to screen and isolate some potential *Trichoderma* strains for further use as biocontrol agents or biopesticides against *S. rolfsii*. This sort of study was worth exploring in order to improve product quality and reduce dependency on chemical fungicides.

2 Materials and Methods

2.1. Materials

2.1.1. Samples

For isolation of different strains of *Trichoderma* spp., rhizosphere soil samples of healthy betelvine plants were collected at different localities of Howrah (i.e. Belpukur, Bagnan etc.), West Bengal, India. *S. rolfsii* (NCIM Cat. No. 1084), the plant pathogen culture was collected from National Collection of Industrial Microorganisms (NCIM), Pune, India.

2.1.2. Culture media

Potato dextrose agar (PDA) was used for fungal culture for this. Peeled potato 200 g, Dextrose 20 g, Distilled water 1000 mL was used and final pH was adjust to 5.6 ± 0.2 at 25 °C. *Trichoderma* selective medium (TSM) media was prepared by using MgSO₄·7H₂O: 0.2 g, K₂HPO₄: 0.9 g, KCl: 0.15 g, NH₄NO₃: 3.0 g, Glucose: 3.0 g, Agar: 20 g, Rosebengal: 0.15 g, Chloramphenicol: 0.25 g, Distilled water: 1000 mL; final pH was adjust to 6.5 ± 0.2 at 25 °C.

2.2. Methods

2.2.1. Isolation of *Trichoderma* spp. from soil

Soil sample (10 g), was dissolved in 90 mL sterilized distilled water and inoculated onto *Trichoderma* selective medium (TSM) agar plates and incubated at 28 ± 2 °C for 7 days. Plates were examined on daily basis and each appeared colony was taken as one colony forming unit (cfu). Individual colonies, isolated from same plates were transferred to freshly prepared potato dextrose agar plates. Pure cultures were maintained on potato dextrose agar slants in sterilized hard glass test tubes and stored at 4 °C for further use.

2.2.2. Soil pH measurement

Soil samples (10 g) containing *Trichoderma* spp. were dissolved in 20 mL distilled water to determine the pH range which is favorable for growth and development of this fungus. Soil pH was measured by pH meter (Elico, Model No. LI 614).

2.2.3. Soil moisture content measurement

Soil samples (20 g) were dried in hot air oven at 121 °C and calculated its weight loss after complete evaporation of water in order to determine moisture range which is favorable for growth and development of *Trichoderma* spp.

Soil moisture content by mass was calculated as per the formula:

Water (%) by mass = (wet mass - dry mass) x100 /dry mass

2.2.4. Identification of *Trichoderma* isolates

Initially *Trichoderma* isolates were recognized on the basis of microscopic characters, conidiophore branching patterns and conidia morphology. For visual observation, isolates were grown on potato dextrose agar for 5 days. Growth rates, changes in medium colour and colony appearance were examined at regular basis. Final identification was done by using recommendations given by Rifai (1969), Bissetts (1984) and Samuel et al. (2002). Further, identification of the two most potent isolates were carried

out by sequence analysis of internal transcribed spacer (ITS)-1 region of rDNA.

2.2.4.1. Genomic DNA isolation, amplification of internal transcribed spacer (ITS)-1 region of DNA and sequencing

Genomic DNA of *Trichoderma* isolate T₃ and T₁₀ were extracted by HipurTM Fungal Genomic DNA Purification Kit (HiMedia, India). Purity of extracted DNA was determined by spectrophotometric method and only pure DNA (A₂₆₀/A₂₈₀=1.8) was used for internal transcribed spacer (ITS)-1 region amplification using universal primers, ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') for forward and primer ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') for reverse. PCR was performed within microcentrifuge tube in Mastercycler Personal with following protocols: initial denaturation for 5 minutes at 94 °C, followed by 35 cycles consisting of denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, elongation at 72 °C for 2 minutes and then cycle was completed by a final elongation step for 5 minutes at 72 °C. PCR products were analyzed by electrophoresis in 1.5% (w/v) agarose gel with ethidium bromide (0.5 µg/mL). PCR products were gel extracted using QIA quick Gel Extraction Kit (Qiagen, Germany). Sequencing were performed in an 8 capillary genetic analyzer (Applied Biosystems, USA) with Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following manufacturer's instruction using the above forward and reverse primers. Obtained nucleotide sequences were assembled using sequence alignment editor program Bioedit (<http://www.mbio.ncsu.edu/Bio-Edit/bioedit.html>) and was compared in GenBank after BLAST searches (Altschul et al., 1997) using EzTaxon-e server (Chun et al., 2007). ITS-1 region (of rDNA) sequences of type strains obtained from EzTaxon-e server, showing more than 97% pair wise sequence similarity, were considered for phylogenetic analysis. A neighbor joining phylogenetic tree was constructed according to Kimura two-parameter model using the MEGA 5 (Tamura et al., 2011) software package (The Biodesign Institute, Arizona, USA). Robustness of tree was conducted by bootstrapping analysis based on 1,000 replicates.

2.2.5. Antagonism studies

To investigate whether antagonism existed between test fungi and pathogen (*S. rolfsii*), 0.5 cm disc of antagonistic fungi and test microorganism (taken from actively growing 3-day-old culture) were placed at centre of potato dextrose agar plates, at 2 cm apart. Three replicates were prepared for each *Trichoderma* isolates. Respective controls were taken as well. Plates were incubated at 30±1 °C for 7 days. Growth pattern and antagonistic colony interaction were studied day by day. Antagonism nature and degree were determined according to the classification of

Skidmore and Dickinsons (Skidmore & Dickinson, 1976) as follows:

Colony interaction	Antagonism types
Mutual intermingling growth	A
Overgrowth by antagonists	Bi
Intermingling growth in which the test fungus under observation has ceased growth and is overgrown by another colony	Bii
Light inhibition	C
Not detected	D

2.2.6. Evaluation of antagonistic potential of *Trichoderma* isolates

Test was conducted to evaluate antagonistic effect of *Trichoderma* isolates against *S. rolfsii* on potato dextrose agar medium by dual culture plate technique (Kucuck & Kivanc, 2003). One mycelia disc (0.5 cm) of individual isolate and one mycelia disc (0.5 cm) of the test pathogen (both taken from actively growing 3-day-old culture) were placed simultaneously 1 cm from the edge of each petriplate at opposite direction. Three replicates were used for each set of experiment. Potato dextrose agar plates with only mycelia disc of *S. rolfsii* was served as control. Plates were incubated at 30±1 °C. Percentage inhibition of *S. rolfsii* was calculated as per the formula cited by Sundar et al. (1995)

$$\text{Inhibition of growth (\%)} = [(A - B) \times 100] / A$$

A = Mycelia growth of the pathogen without *Trichoderma* spp. (control).

B = Mycelia growth of the pathogen in presence of *Trichoderma* spp.

2.2.7. Effect of non-volatile compounds produced by antagonist(s) on radial growth of *S. rolfsii*

Production of non-volatile substances by selected *Trichoderma* isolates against test pathogen was studied as per method described by Dennis & Webster (1971). Each *Trichoderma* isolate was inoculated in 100 mL sterilized potato dextrose broth in 250 mL conical flasks and incubated at 30±1 °C on a rotary shaker with intermittent shaking at 150 rpm for 15 days. Later culture was filtered through Whatmann No.1 filter paper and centrifuged at 10000 rpm for 10 minutes for removing mycelial mats. After sterilization by passing through 0.4 µm biological membrane filter, filtrate was added to melted potato dextrose agar medium (at 40±2 °C) to make concentration of 5%, 10%

and 15% (v/v) in petriplate. Filtrate amended potato dextrose agar containing plates were inoculated with 0.5 cm mycelial disc of *S. rolfsii* at the centre of petriplate and incubated at 30 ± 1 °C for 5 days or until colony reached edge of petriplate. There were three replicates for each treatment. Two types of control, plate without any kind of culture and only pathogen culture without filtrate, was taken. Radial growth of pathogen was taken daily and percent inhibition was calculated by using following formula.

$$\text{Inhibition of growth (\%)} = [(C - T) \times 100] / C$$

C = Mycelia growth of the pathogen (*S. rolfsii*) without filtrate (control).

T = Mycelia growth of the pathogen in presence of filtrate

2.2.8. Effect of volatile compounds produced by antagonist(s) on radial growth of *S. rolfsii*

Selected isolates were tested for production of inhibitory volatile metabolites to seize growth of *S. rolfsii*. "Inverted plate technique" by Dennis & Webster (1971) was used for this study. Agar discs (0.5 cm in diameter) were cut from actively growing (3-day-old) *Trichoderma* isolates and placed on centre of a potato dextrose agar plate. Similarly, 0.5 cm mycelial disc of *S. rolfsii* was inoculated at the centre of separate potato dextrose agar plates of same diameter. Subsequently, upper lids of both plates were removed and plate containing *S. rolfsii* was inverted over the plate containing *Trichoderma* isolate. Then, both plates were sealed at junction with parafilm. Control plate was maintained with *Sclerotium* plate inverted and sealed over an uninoculated potato dextrose agar plate. Each treatment was replicated thrice. All plates were incubated at 30 ± 1 °C for 7 days. Observations on radial growth of pathogen were taken regularly and percent inhibition was calculated by comparing with control plate as described earlier.

2.2.9. Activity of *Trichoderma* isolates on sclerotia germination in liquid medium

Sclerotia of similar size and shape from a 15-day-old culture plate were inoculated in 250 mL potato dextrose broth (in Erlenmeyer flasks), containing culture filtrate of different strains of *Trichoderma* isolates. Three flasks for each *Trichoderma* filtrate were maintained. Sclerotia, inoculated in sterilized saline water were served as control. Sclerotia were removed from each flask at regular interval and plated onto fresh potato dextrose agar plates after surface sterilization with 0.2% HgCl₂. The process was repeated till no sclerotia remained viable in treatment flasks.

3 Results

3.1. Isolation of *Trichoderma* spp.

Eleven *Trichoderma* spp. was isolated from twenty four soil samples collected from different betelvine agricultural fields in Bagnan, Howrah. Most of these isolated colonies were plumose with white floccose surface along with scattered green patches. Pigment was secreted and diffused into medium during growth of isolates.

3.2. Soil pH and moisture measurement

Trichoderma spp. showed its ability to grow and develop in different pH ranging from 4.45 to 7.97, with mean soil pH value of 6.21 (Table 1). This demonstrated that *Trichoderma* spp. is distributed widely in various soil conditions with different pH values. It can grow and sustain also in various humid conditions ranging from 18.85-47.34% with average moisture content of 32.34%.

Table 1 pH values of soils containing *Trichoderma* spp.

Isolate No.	pH value	Moisture content (%)
1	4.45	18.85
2	5.18	20.75
3	4.67	20.75
4	7.97	47.34
5	6.7	31.23
6	6.53	34.56
7	5.98	27.91
8	7.87	43.35
9	6.42	43.45
10	5.93	34.12
11	6.61	33.47
Mean	6.21	32.34

3.3. Identification of isolates

Among eleven isolates, seven isolates (Isolate T₁, T₃, T₄, T₅, T₇, T₉ and T₁₁) were preliminary identified as *T. harzianum* and rest of the isolates (Isolate T₂, T₆, T₈ and T₁₀) were *T. viride* (Table 2). Further, sequence analysis result of internal transcribed spacer (ITS)-1 region of DNA confirmed isolate T₃ to be *T. harzianum* and isolate T₁₀ as *T. viride* (Figure 1).

Table 2 Characterization and identification of *Trichoderma* isolates

Isolate No.	Macro / Microscopic characteristics	Identified isolates
T ₁ , T ₃ , T ₄ , T ₅ , T ₇ , T ₉ , T ₁₁	At early stage, whitish to greenish mycelia appeared. Gradually deep green colour developed in central part and extended to periphery of plate. Finally, it appeared a dull green colour. Mostly spherical, smooth conidia produced on conidiophore.	<i>Trichoderma harzianum</i>
T ₂ , T ₆ , T ₈ , T ₁₀	Colony was observed to be whitish at early stage (3-4 days). Gradually from light green, dark green coloured mycelia mat exhibited at the central part of PDA plate. Conidiophores were erect, compact, woolly pectinately branched. Conidia was hyaline, subglobose, curve shaped, oval and smooth walled.	<i>Trichoderma viride</i>

Figure 1 Phylogenetic tree for *Trichoderma* isolates T₃ and T₁₀ using neighbour-joining method (Among closely related type species showing more than 97% sequence similarity. Bootstrap values (based on 1000 replicates) >50% are given at branch points. Accession numbers are given in parentheses. Bar: 2 substitutions per 1000 nucleotide position).

3.4. Antagonism study

Eleven isolates were tested to find out their antagonistic potential and nature of colony interaction against test pathogen, *S. rolfisii*. Three *T. harzianum* isolates (T₃, T₄ and T₁₁) showed Bi type of interaction (Table 3), antagonist inhibited growth of *S. rolfisii* by overgrowing. Whereas, *T. viride* isolate T₂ and T₁₀ showed Bii type of interaction. From laboratory study, it was observed that among the test fungi, *T. harzianum* isolate T₃, T₄ and T₁₁ and *T. viride* isolate T₂ and T₁₀ were mainly effective against *S. rolfisii*.

Table 3 Types of colony interaction of the antagonists with test fungus (*S. rolfisii*)

Isolate No.	Name of the antagonist	Type of interaction
T ₁	<i>T. harzianum</i>	C
T ₂	<i>T. viride</i>	Bii
T ₃	<i>T. harzianum</i>	Bi
T ₄	<i>T. harzianum</i>	Bi
T ₅	<i>T. harzianum</i>	C
T ₆	<i>T. viride</i>	C
T ₇	<i>T. harzianum</i>	C
T ₈	<i>T. viride</i>	C
T ₉	<i>T. harzianum</i>	C
T ₁₀	<i>T. viride</i>	Bii
T ₁₁	<i>T. harzianum</i>	Bi

In dual culture plate technique, pairing of *Trichoderma* isolates with *S. rolfii* gave rise to growth reduction of test plant pathogen. *T. harzianum* isolate T₃ produced highest inhibition of 71.67%, while *T. viride* isolate T₁₀ showed an inhibition of 67.23%. Reduction in radial growth of *S. rolfii* by *T. harzianum* and *T. viride* differed significantly ($P > 0.05$) from that of the growth of *S. rolfii* without antagonist as shown in Figure 2.

Figure 2 Percentage growth inhibition of *S. rolfii* paired with *Trichoderma* isolates

3.5 Effect of non-volatile compounds produced by antagonist(s) on radial growth of *S. rolfii*

Among *Trichoderma* isolates, irrespective of concentration and time of incubation, *Trichoderma* isolate T₁₀ culture filtrate was found to be the most effective. At 15% concentration level, it can totally suppress mycelia growth. *Trichoderma* isolate T₃ recorded radial growth of 1.7 cm on 8th day of incubation in comparison to control, representing 81.52% mycelia growth rate inhibition at 15% (v/v) concentration level. In case of isolate T₁₁, *S. rolfii* showed highest radial growth (7.12 cm) on 8th day of incubation, indicating the least inhibition (Table 4).

3.6. Effect of volatile compounds produced by antagonist(s) on radial growth of *S. rolfii*

Among five different *Trichoderma* isolates, *T. viride* isolate T₂ showed maximum potential in volatile antagonistic compound production with 53.26% radial growth inhibition on 8th day of incubation (Table 5). Isolate T₃ followed it with 52.17% of growth inhibition. Except isolate T₁₀, other four *Trichoderma* isolates (T₃, T₄, T₁₁ and T₂) had potential to produce volatile compound to inhibit *S. rolfii*.

Table 4 Effect of non-volatile compounds produced by antagonist(s) on radial growth of *S. rolfii*

Incubation period	Trichoderma filtrate concentration (%) in medium (v/v)					
	5%	Inhibition (%)	10%	Inhibition (%)	15%	Inhibition (%)
Isolate T ₃						
4 days	4.34±0.01	15.73±0.04	3.10±0.04	39.81±0.03	1.20±0.03	76.70±0.02
8 days	8.3±0.01	9.78±0.02	6.40±0.03	30.43±0.01	1.70±0.01	81.52±0.04
Isolate T ₄						
4 days	4.7±0.03	8.74±0.01	3.90±0.02	24.27±0.01	3.10±0.03	39.81±0.02
8 days	8.6±0.01	6.52±0.03	6.70±0.03	27.17±0.02	5.20±0.02	43.48±0.01
Isolate T ₁₁						
4 days	4.9±0.03	4.85±0.01	4.20±0.01	18.45±0.01	3.87±0.01	24.85±0.03
8 days	8.6±0.03	6.52±0.02	7.30±0.02	20.65±0.03	7.12±0.03	22.61±0.04
Isolate T ₂						
4 days	4.8±0.04	6.80±0.03	4.00±0.03	22.33±0.02	3.40±0.03	33.98±0.01
8 days	8.5±0.03	7.61±0.01	6.90±0.04	25.00±0.01	5.90±0.02	35.87±0.03
Isolate T ₁₀						
4 days	2.9±0.03	43.69±0.01	1.05±0.03	79.61±0.01	0	100.00
8 days	4.54±0.01	50.65±0.02	1.55±0.02	83.15±0.04	0	100.00

*Each value is an average of 3 replicate samples ± standard deviation in cm; Control growth @ Day 4: 5.15±0.02 cm and Day 8: 9.20±0.01 cm

Table 5 Effect of volatile compounds produced by antagonist(s) on the radial growth of *S. rolfii*

Time	Effect of volatile compounds	
	Mycelia growth (cm)	Inhibition (%)
Isolate T ₃		
4 days	3.15±0.02	43.69±0.04
8 days	4.95±0.03	52.17±0.05
Isolate T ₄		
4 days	3.00±0.01	41.75±0.03
8 days	4.70±0.02	48.91±0.02
Isolate T ₁₁		
4 days	2.90±0.01	38.83±0.02
8 days	4.40±0.02	46.20±0.01
Isolate T ₂		
4 days	2.80±0.02	45.63±0.024
8 days	4.30±0.01	53.26±0.05
Isolate T ₁₀		
4 days	5.10±0.02	0.97±0.04
8 days	9.10±0.03	1.09±0.03

*Each value is an average of 3 replicate samples ± standard deviation, Control Growth @ Day 4: 5.15±0.02 and Day 8: 9.20±0.01

3.7. Activity of *Trichoderma* isolates on sclerotia germination in liquid medium

Results of viability test are given in Figure 3, which shows that isolate T₁₀ was the most effective and isolate T₁₁ was the least effective antagonist among tested *Trichoderma* isolates. All the five antagonistic isolates suppressed sclerotial germination and completely killed sclerotia within 20 days; only isolate T₁₁ required 25 days. After 25 days, no sclerotia were found viable in treatment assay flasks; however 100% sclerotia remained viable in control flasks.

4 Discussion

In this study, two *Trichoderma* species viz. *T. harzianum* (seven strains) and *T. viride* (four strains) were isolated from soil samples. Finding of this study suggested that *Trichoderma* is very common in soil and root ecosystem, hence can be easily isolated from soil and other organic material. Ubiquitous saprobe nature of *Trichoderma* have also been reported earlier by Harman et al. (2004) and Zeilinger & Omann (2007).

In dual culture plate experiment, isolated *Trichoderma* strains were concurrently paired with *S. rolfii* on potato dextrose agar plate for 7 days to observe pattern of antagonism and compare ability of isolated *Trichoderma* strains to compete with pathogen fungi for space and nutrients. From results, it was clear that *T. harzianum* isolate T₃ had the highest potential to inhibit *S. rolfii*

Fig. 3 Activity of *Trichoderma* spp. on sclerotia germination in liquid medium

(71.67% inhibition) followed by *T. viride* isolate T₁₀ (67.23% inhibition). Results indicated that isolate T₃ and T₁₀ can parasitize *S. rolfsii* hyphae by means of penetration and growth inside hyphae of *S. rolfsii*. These findings showed parity with the observations made by Nagamma & Nagaraja (2015). Earlier, Howell (2003) also suggested substantial antagonistic effect of *T. harzianum* on mycelia growth of *S. rolfsii*. As reported in present study, inhibition of *S. rolfsii* in dual culture plate experiment can be attributed to more rapidly growing ability of *Trichoderma* species and secretion of toxic extra cellular compounds such as antibiotics and cell wall degrading enzymes, i.e. β -1,3-glucanases, chitinases and proteases (Howell, 2003; Gajera et al., 2012). During mycoparasitic activity, these enzymes lyse hyphal cell wall of pathogens. According to study by Samieto et al., (2010), antagonism by both *T. harzianum* and *T. viride* strains is achieved through a multi-factorial process that required synergistic contribution of several mechanisms including entwining hyphae, spores attachment to its host, growing inside host conidia and subsequently death of host conidia.

Mycelia growth inhibition of *S. rolfsii* by isolated *Trichoderma* culture filtrate in liquid medium varied from 22.61% to 100%. This kind of result is analogous to study by Sivasithamparam & Ghisalberti (1998), which indicated that different species of same family and different strains of same species often can inhibit pathogens in significantly diverse manner as secondary metabolites express individuality of species in chemical terms. Mycelial growth inhibition by non-volatile compounds could be due to vacuolation, granulation, coagulation and lastly disintegration of cell wall by metabolites produced by *Trichoderma* isolates.

T. viride isolate T₂ inhibited growth of *S. rolfsii* mostly followed by Isolate T₃ by means of volatile compound production. In this context, Shaigan et al. (2008) stated the effect of volatile metabolites produced by *T. viride*, *T. harzianum* and *T. longibrachiatum* on *S. rolfsii* with mycelia growth inhibition of 60.8, 58.8 and 58.4% respectively, just as occurred in this bioassay. Volatile compounds were different among isolated strains of *Trichoderma* and mycelia growth inhibition by volatile compounds was prominently heterogeneous, even in strains of same species. Variable production of volatile metabolites by *Trichoderma* strains was reported by Amin et al. (2010). They even mentioned production of different metabolites by particular strain in different stages of development in dissimilar growth conditions.

From viability test, it was evident that all *Trichoderma* isolates could suppress sclerotia germination to a large extent. Isolate T₁₀ had the most, while T₁₁ had the least potential as sclerotia germination suppressing bio agent. Within a span of 25 days from treatment, viable sclerotia had no traces in flask.

Conclusion

Two of the five selected *Trichoderma* isolates could effectively inhibit growth of *S. rolfsii* under laboratory conditions. *S. rolfsii* inhibiting ability of *Trichoderma* isolates can be explained taking into account of their capability to compete and exhibit mycoparasitism. These results suggested possible promising application of these isolated *Trichoderma* spp. against different phytopathogenic fungi.

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Conflict statement:

The authors certify that they have no conflict of interests.

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