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# Exploration of Native *Trichoderma* Species as a Bio-control Agent against *Sclerotinia sclerotiorum* Pathogen in Ginger Rhizomes (*Zingiber officinale* Roscoe)

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***Abstract*—**The study was conducted at IAMS (Pvt) Ltd, Alawwatta, Wariyagoda, Alawwa to identify native *Trichoderma* species to serve as bio-control agents against *S. sclerotiorum* pathogen in ginger cultivation. Pathogen was isolated from infected ginger rhizomes collected from farm fields in Anuradhapura and identified based on the morphology of fungal colonies and sclerotia production on Potato Dextrose Agar (PDA). *Trichoderma* species were isolated from different ecological habitats in Nuwara Eliya district using pour plate technique and five isolates (T1, T2, T3, T4 and T5) were recognized using colony color, growth rate of mycelia and shape of the conidia. Then five *Trichoderma* isolates were subjected to in vitro screening against pathogen by comparing percentage growth inhibition (GI %). Results revealed that all the five *Trichoderma* isolates inhibited the growth of *S. sclerotiorum* under in-vitro condition. Isolates T2, T3, T4 and T5 showed significantly higher growth rate after 3 days of incubation on PDA compared to T1. Isolates T1 and T2 showed significantly higher GI % of *S. sclerotiorum* compared to isolate T3, T4 and T5. Hence, *Trichoderma* isolatesT1 and T2 can be developed as bio-control agents against *S. sclerotiorum* of ginger cultivation in Sri Lanka with further studies to confirm the bio-efficacy under field conditions.

***Keywords*—**Bio-control agents, Growth inhibition, Pathogen, *Sclerotinia sclerotiorum*, *Zingiber officinale* Roscoe.

## I. Introduction

Ginger (*Zingiber officinale* Roscoe) is a widely cultivated herb all over the world and consumed for culinary and medicinal purposes (Kiyama, 2020). It is grown as a spice, food, flavoring and supplement agent because of its favorable characters including nutrients, aroma, pungency and phyto-chemical activity (Baliga *et al*., 2011). The ginger plants have underground stems, commonly called as rhizomes, which is used for vegetative propagation and food storage (Ravindran *et al*., 2005). This crop is grown in wet and intermediate zones of Sri Lanka and widely farmed as an inter-crop in coconut plantations (Ginigaddara, 2018).

However, ginger quality and yield are severely affected by various diseases and plant pathogens (Ayodele *et al*., 2018). Among the pathogens, *Sclerotinia sclerotiorum*, Fusarium solani, and Rhizoctonia solani are all abundant in nature and have a wide spectrum of hosts (Manandhar *et al*., 2019). *S. sclerotiorum* is the causative agent of various diseases such as soft rot, rhizome rot, yellow rot and sclerotium rot in ginger (Rakesh *et al*., 2013). The survival structure of this pathogen is called sclerotia and it acts as the main inoculum source in the field (Bolton *et al*., 2006). The sclerotia germinate and infect the host plants when conditions are favorable. It secretes multiple pathogenic factors, degrading and macerating the plant cell wall and tissue. The most common symptoms of this pathogen attack are unusual water-soaked lesions and distinctive white-cotton like mycelium in leaves, roots and petioles (Smolinska *et al*., 2018).

Though chemical pesticides significantly contribute in controlling pest and disease, concerns have been raised about their demerits on human health and environment. As an alternative, biological control method is viewed as environmentally friendly and avoids the threats posed by chemical fertilizers (Kumar *et al*., 2017). A bio-controlling method is the use of natural organisms to control a pest species (Sharma *et al*., 2013). *Trichoderma* spp. is the widely studied bio-control agent and has been used against many soil-borne pathogens specially fungi (Mukherjee *et al*., 2012). It can be isolated from soil, plant root, decomposing woods or other organic debris (Motlagh *et al*., 2021). They generate antibiotics and other cell wall dissolving enzymes that are lytic and harmful to a variety of fungus. However, not all *Trichoderma* strains can control all the pathogens successfully, therefore finding the *Trichoderma* strain with bio-control capability is critical. Native *Trichoderma* spp. can have substantial bio-controlling ability since they are adapted to the environment whereas exotic *Trichoderma* spp. can have problems of climatic resilience and soil colonization (Kamala and Devi 2012). Therefore, the aim of this research

was to identify native *Trichoderma* spp. isolates with bio-control ability against *S. sclerotiorum* a rhizome rot pathogen of ginger in Sri Lanka.

## II. Materials and Methodology

### A. Research Area

This study was conducted at the microbiology laboratory of IAMS (Pvt) Ltd, Alawwatta, Wariyagoda, Alawwa.

### B. Collection of infected ginger rhizomes and surface sterilization

Plant pathogenic fungi were obtained from various infected ginger rhizomes collected from farmers in Anuradhapura district. Ginger rhizomes were washed carefully using tap water to get rid of adhering soil particles and then rinsed with sterilized water for three times. Samples were put in clean plastic bags and stored at room temperature. Each plant rhizomes were cut into small pieces and surface sterilization was done by immersing the samples with 70 % (v/v) ethanol for 1 minute, rinsing three times with sterilized water, soaking in 0.5 % sodium hypochlorite solution (NaOCl) for 5 min and rinsed 6 times in sterilized distilled water (Schulz *et al*., 1993). After that the samples were dried on sterilized filter paper for 12 hours. All preparations were carried out inside the laminar airflow cabinet (Lab Fume Laminar Air Flow Hood). The pieces were placed aseptically in potato dextrose agar (PDA) plates and incubated for 2-4 days at room temperature.

### C. Isolation of S. sclerotiorum pathogenic fungi from ginger rhizomes

*S. sclerotiorum* were isolated using PDA medium and maintained on it for long-term use after drying at 4 °C. Pathogen was purified using series of sub culturing and agar plugs containing actively growing mycelium were transferred to PDA plates and then incubated 2-3 days at room temperature (Singh, 1991).

### D. Isolation of Trichoderma species from soil samples

Different ecological habitat areas namely, virgin forest soil, pine plantation, tea plantation, banana cultivation and ginger cultivation in the Nuwara Eliya district were selected to obtain the soil samples. After the collection, the soil samples were air-dried in biosafety cabinet and stored under 25 °C for further usage. *Trichoderma* species were isolated by using pour plate technique method (Kaur *et al*., 2014). Using sterilized water, five-fold serial dilutions of each soil sample were made and 0.5 ml of the diluted sample was spread onto the surface of *Trichoderma* species-specific media. Plates were incubated at room temperature for 96-144 h (4 – 6 days) until the morphologically different colonies appeared. Subsequently, individual colonies were isolated from sample plates, with unique colony being re-isolated onto a new PDA plate. Several sub culturing were performed to isolate pure *Trichoderma* colonies and they were preserved at 4 °C. Accordingly, five fungal strains (T1, T2, T3, T4 and T5) were isolated from different samples of soil.

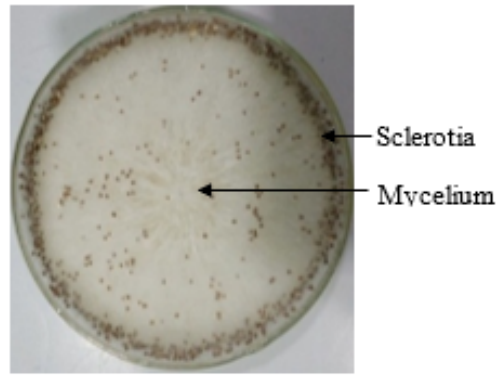


Figure 1: Growth of *S. sclerotiorum* on PDA medium after 7 days of inoculation

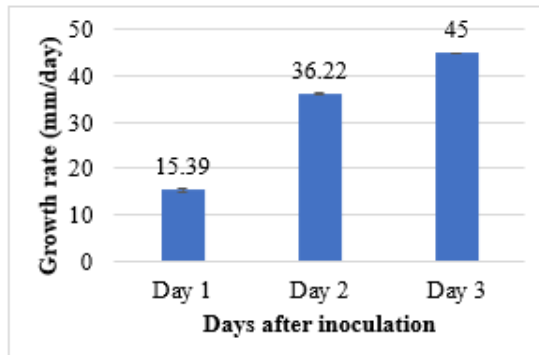


Figure 2: Growth rate of *S. sclerotiorum* in three days on 9 mm petri plate. Vertical bars represent ± standard error.

### E. In- vitro screening of Trichoderma isolates against S. sclerotiorum

Isolated *Trichoderma* species were subjected to in vitro screening against *S. sclerotiorum* by dual culture plate technique (Parizi *et al*., 2012). The dual culture was developed by placing the isolated 5 mm diameter disc of *Trichoderma* colony diametrically opposite to the *S. sclerotiorum* pathogen on PDA plate. Each *Trichoderma* spp. treatment consisted of 3 replicates and monoculture plates of *Trichoderma* and *S. sclerotiorum* were maintained as controls. All the plates were incubated at room temperature and observed daily for three days.

$$GI(\%) = 100 - 100 \times (\frac{R2}{R1}) \quad (1)$$

$GI =$ Inhibition of vegetative growth of *S. sclerotiorum*
$R1 =$ Radius of the *S. sclerotiorum* colony in the control plate
$R2 =$ Radius of the *S. sclerotiorum* colony in the dual culture plate

Accordingly above formula, higher GI % mean that *Trichoderma* isolate more effectively control pathogen

### F. Data Collection and analysis

The growth rate (mm/day) of *S. sclerotiorum* and *Trichoderma* was obtained daily by measuring the radius of each

growing fungal colony using a ruler in two perpendicular direction without opening the petri dishes until the plates are completely colonized. Growth inhibition was calculated using the following formula (Keshvachandran *et al*., 2007),

### G. Statistical analysis

All of the data were analyzed using one-way analysis of variance (ANOVA) in SPSS software and Tukey's post-hoc test was used to determine whether there were significant differences between individual means at $p<0.05$.

## III. Results and Discussion

### A. Growth of *S. sclerotiorum* pathogen

*S. sclerotiorum* were identified and confirmed by comparing the morphological characters of the isolated fungi with previously published records (Smolinska and Kowalska, 2018). White color mycelium development was observed during the culturing on PDA and it took 3 to 4 days to fully cover the surface of the petri-plates (9 cm diameter) (Figure 1). After 4-7 days of mycelium formation, the survival structure sclerotia began to develop. Initially, protuberances appeared in fluffy white color that later grew into tiny circular dark- brown sclerotia. The number of sclerotia produced on PDA in culture ranged from 1201-1999 per petri dish, and the diameter was in the range of 0.8 mm to 1 mm. Growth rates of *S. sclerotiorum* were 15.39 mm/day, 36.22 mm/day and 45 mm/day after 1st, 2nd and 3rd days of inoculation (Figure 2).

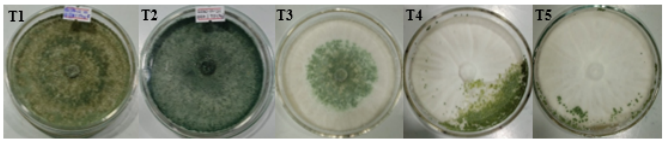


Figure 3: Morphological features of different isolates of *Trichoderma* on PDA

### B. In-vitro screening of *Trichoderma* spp. Isolates against to *Sclerotinia sclerotiorum*

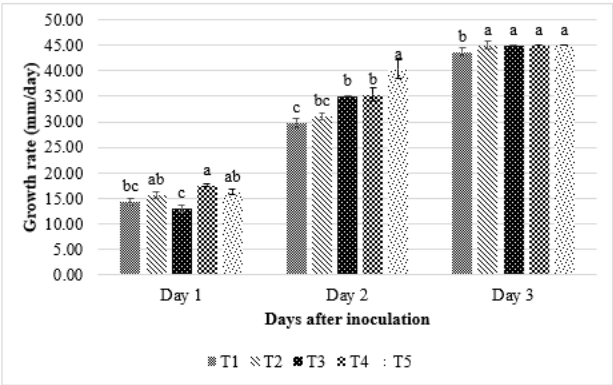


Figure 4: Growth rate of *Trichoderma* isolates on PDA during the incubation period. Vertical bars represent ± standard error. Bars with the same letter are not significantly different at Tukey's posthoc test at p = 0.05.

Table I: Morphological characters of different *Trichoderma* spp on PDA after 3 days of incubation.

| Trichoderma spp. isolates | Colony color (Upper) | Revers Colony color | Shape of conidia |
|---|---|---|---|
| T1 | Yellowish green | Amber | Oval |
| T2 | Bright green | Pearl white | Ellipsoidal |
| T3 | Dark green | Off-white ash | Ellipsoidal |
| T4 | Yellowish green | White | Spherical |
| T5 | Greenish yellow | White wrinkle | Oval |

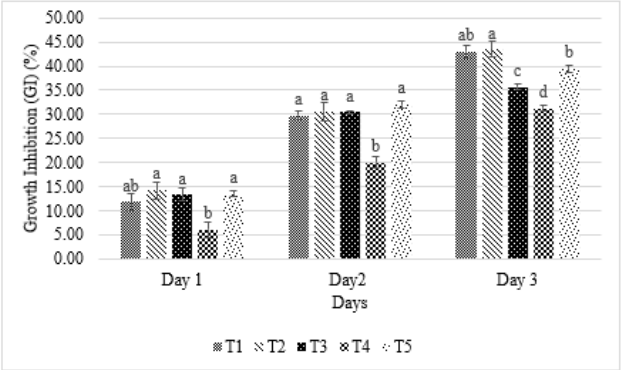


Figure 5: Growth inhibition of *S. sclerotiorum* by isolates of *Trichoderma* spp. Vertical bars represent ± standard error. Bars with the same letter are not significantly different at Tukey's posthoc test at p = 0.05.

*Trichoderma* suppress the mycelia growth of *S. sclerotiorum* isolate by overgrowing on it. Screening of different isolates of *Trichoderma* against *S. sclerotiorum* were performed by dual culture plate technique using PDA media (Figure 6). The inhibition zones were observed between the *Trichoderma* and *S. sclerotiorum* . All the five *Trichoderma* isolates were found to be significantly effective in inhibiting the growth of *S. sclerotiorum* in-vitro. Significantly higher GI % of *S. sclerotiorum* was observed in T2 (14.28 %), T3 (13.29 %) and T5 (13.55 %) in day 1 meanwhile, isolates T1 (29.7 %), T2 (30.56 %), T3 (30.44 %) and T5 (32.02 %) showed significantly higher GI % in day 2. In day 3 isolates T2 (43.45 %) and T1 (43.04 %) showed significantly higher GI % of *S. sclerotiorum* compared to isolates T3, T4 and T5 (Figure 5).

*S. sclerotiorum* is the most devastating soil borne fungal pathogens which has a very wide range of host and causes severe economic losses in crops (Lalfakawma *et al*., 2021).

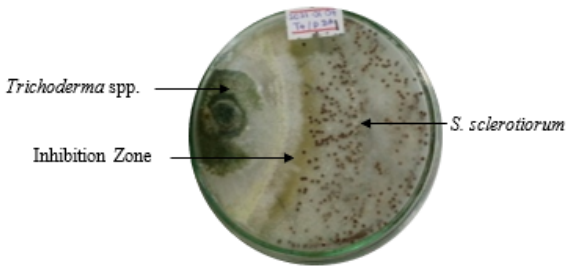


Figure 6: Dual culture plate of *Trichoderma* spp. against *S. sclerotiorum*

Biological control of plant diseases has received a lot of attention recently. Unlike chemical control, this approach is effective and long-lasting. *Trichoderma* is a well-known bio-control agent which directly parasitize sclerotia of fungal pathogens and produce antibiosis by releasing hydrolytic enzymes and peptides. It competes with pathogens for nutrients and induce systemic plant defenses (Ousley *et al*., 1994). However, the degree of control depends on *Trichoderma* species and strains (Geraldine *et al*., 2013). This had been proved in the present study as well that different *Trichoderma* isolates showed significant variations in the biological control of *S. sclerotiorum* .

## IV. Conclusions

Five different *Trichoderma* isolates were extracted from different ecological habitats in the Nuwara Eliya district. Causal pathogen of ginger rhizome rot was identified as *S. sclerotiorum* . Among the tested *Trichoderma* isolates, two isolates were shown significantly higher growth inhibition of *S. sclerotiorum* compared to others under in vitro conditions. The findings of this study showed that different *Trichoderma* isolates extracted from different ecological habitats in the Nuwara Eliya district had the ability to control *S. sclerotiorum* pathogen i.e. causal agent of rhizome rot of ginger cultivation in Sri Lanka. However, further studies are necessary to get conclusive results of bio efficacy of *Trichoderma* as bio control agent against fungal pathogen *S. sclerotiorum* under the greenhouse and field conditions.

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