Original Research Article

Varietal Reaction and Efficacy of *Trichoderma* in Managing Tomato Collar Rot Disease Caused by *Sclerotium rolfsii*

ABSTRACT

The research was conducted to study the morphology and pathogenicity of *Sclerotium rolfsii* isolates, to evaluate the responses of tomato varieties to collar rot disease, and to select the effectiveness of *Trichoderma* isolates and efficacy of some fungicides in controlling on this disease. The experiments were laid out in completely randomized design and carried out at the Department of Plant Pathology, Yezin Agricultural University (YAU) during February, 2024 to February, 2025. Total seven isolates of *S. rolfsii* were obtained from the tomato fields of seven villages in Zeyarthiri Township, Nay Pyi Taw Union Territory and named the isolates as SR1, SR2, SR3, SR4, SR5, SR6 and SR7. Their pathogenicities were tested on the tomato variety, Kyaukme Gaung Seine. Except SR7 isolate, other six isolates caused 100% seedling mortality. After inoculation of ten tomato varieties with the *S. rolfsii* isolate; SR5, the variety; Red Diamond115 was moderately susceptible while the rest were susceptible to the disease. According to the molecular characterization based on PCR with species specific primers, HAR 220FP5 and HAR220RP5 for *Trichoderma harzianum* and VIRI900FP7 and VIRI900RP7 for *Trichoderma viride,* the commercial *Trichoderma* isolate (DAR-Tri.sp) was identified to be *T. harzianum* and the four *Trichoderma* isolates from YAU were diagnosed to be *T. viride*. When the effectiveness of *Trichoderma* isolates on mycelial growth of *S. rolfsii* were tested, YAU-Tri.sp4 showed the highest inhibition percent among the tested five *Trichoderma* spp.. Also, it had promoted tomato plant growth and controlled the collar rot disease (84%) compared to *Trichoderma* uninoculated plants. Therefore, YAU *Trichoderma* isolate, YAU-Tri.sp4 could be used as a potential biocontrol agent for the management of tomato collar rot disease.

Keywords: Collar rot disease, *Sclerotium rolfsii*, Tomato, *Trichoderma*

1. INTRODUCTION

Tomato is one of the important commercial vegetable crops in Myanmar, where it is cultivated on approximately 101,000 ha and yield was 11.25 MT ha^{-1} in 2022-2023, it is mainly cultivated in Mandalay, Magway, Sagaing, Bago Regions, and Southern Shan State (Ministry of Agriculture, Livestock and Irrigation [MOALI], 2023). Tomato fruits are rich in minerals, vitamins, essential amino acids, carbohydrates as well as dietary fibers. It contains a diverse range of vitamins B, and C and antioxidant compounds. Its main antioxidant compounds are phenolic compounds, vitamin C, vitamin E, carotenoids such as β-carotene, and especially lycopene (Szabo et al., 2021). According to data from FAOSTAT (2022), the world average yield of tomatoes was 37.1 metric tons per hectare (MT ha^{-1}). Soil-borne and

foliar fungal diseases are major limiting factors for tomato production (~~Pavan~~ Kumar, 2018). Soil-borne fungal pathogens such as *Sclerotium rolfsii*, *Pythium* spp., and *Rhizoctonia solani* infect the tomato crop causing damping off disease and is becoming a potential threat to its cultivation (Prasad ~~, Vidya Sagar, Rao & Koteswar~~et al., 2017). Among the soil-borne fungal diseases, the phytopathogenic fungi, *Sclerotium rolfsii*, which causes foot rot or collar rot in tomatoes is a serious disease (Brindhadevi ~~, Thiruvudainambi, & Theradimani~~et al., 2021). The pathogen causes different types of diseases such as collar rot, sclerotium wilt, stem rot, charcoal rot, seedling blight, damping-off, foot rot, stem blight, and root rot in many economically important agriculture and horticulture crops (Chowdary~~, Jameema, & Charishma~~et al., 2024). Biological control agents, such as *T.richoderma harzianum, T.~~richoderma~~ viride*, and certain mycorrhizal fungi have been shown to provide some control of *S. rolfsii* (Garcia-gonzalez ~~, Hansen, Strawn & Rideout~~et al., 2021). The genus *Trichoderma* consists of anamorphic fungi isolated primarily from soil and decomposing organic matter, with teleomorphs, belonging to the ascomycete genus *Hypocrea*. *Trichoderma* species are being used alone or in combination with compatible chemicals for the control of several soil-borne diseases such as collar rot, root rot, wilt, etc. (Adhikari ~~, Shrestha, Manandhar & Marahatta~~et al., 2022). *Trichoderma* spp. employs numerous strategies for the protection of plants against plant pathogens which comprise mycoparasitism, competition, antibiosis, parasitism, and the induction of systemic resistance (Benítez ~~, Rincón, Limón & Codón~~et al., 2004). Now, collar rot disease caused by *S. rolfsii* become serious on various crops including tomatoes and there is little awareness of this disease. As the pathogen is soil-borne and causes diseases on several kinds of crops, it is necessary to find out effective control measures. At present, resistant varieties and biocontrol agents (*Trichoderma*) are widely practiced by farmers. Therefore, finding out the resistance varieties to this disease and effective biocontrol agents are important research works. However, there is limited information regarding the resistance level of tomato varieties to *S. rolfsii* and the application of a biocontrol agent (*Trichoderma*) for controlling tomato collar rot disease.

Therefore, this research was conducted to determine the morphological characters and pathogenicity of *S. rolfsii* isolates causing tomato collar rot disease, to evaluate the responses of tomato varieties to collar rot disease, to identify the *Trichoderma* isolates from Yezin Agricultural University, and to evaluate the effectiveness of *Trichoderma* isolates against the tomato collar rot *in vitro* and *in vivo.*

2. MATERIALS AND METHODS

2.1 Collection of disease sample, isolation, and identification of the pathogen

All tomato collar rot specimens were collected from seven villages (Shwebe, Shwethinbyu, Shwebaho, Sibintharyar, Kanoo, Montaekhwin, and Yezin) located in Zeyarthiri Township, Nay Pyi Taw Union Territory. The infected portions of diseased plants were thoroughly washed and cut into small pieces (5mm) in diameter and surface sterilized with 5% (w/v) sodium hypochlorite (NaOCl) solution for 1 min and then in 70% (v/v) ethanol for a further minute. Then, they were rinsed thrice in sterile distilled water, and dried on sterilized filter paper. The samples were placed aseptically into a 9 cm diameter petridish containing 2% (w/v) water agar and sub-cultured on Potato Dextrose Agar (PDA; 200g: 20g: 20g in 1L water) medium and incubated for 3-5 days at room temperature ($27 \pm 2°C$). After that it was examined for morphological characters, such as hyphal diameter, growth rate (mm day^{-1}), colony morphology (fluffy or

compact), and sclerotia size.The diameter of the sclerotia was measured by using a USB digital microscope (MicroCapture, China). The pathogen was identified based on its mycelial and sclerotial characters according to the descriptions of Mullen (2001).

2.2 Pathogenicity test

The pathogenicity of seven *S. rolfsii* isolates was tested on the tomato variety, Kyaukme Gaung Seine which was the most cultivated variety in disease sample collected areas. Seeds of the test variety were obtained from the Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw. A mycelial disc measuring 5 mm in diameter was taken from 3 days old fungal colony of each isolate and placed at the center of 9 cm petridish containing PDA media. They were incubated for 3 days. Tomato seeds were surface sterilized in 5 % NaOCl for 1 minute and rinsed with sterilized water 3 times. For inoculation, PDA petridishes containing the mycelial mats were overlaid with 30 g of sterilized soil and then ten surface sterilized seeds were sown in the petridishes. The PDA petridishes without fungal growth were also overlaid with sterilized soil and ten surface sterilized seeds were sown as a control. The experiment was laid out in a completely randomized design (CRD) with four replications. At 7 days after incubation, the seedling stands in the inoculated and uninoculated petridishes were recorded and the seedling mortality percent was calculated with the following formula given by Kataria & Grover, (1976) .

$$\text{Seedling mortality (\%)} = 100 - [\frac{\text{Seedling stand in inoculated soil}}{\text{Seedling stand in uninoculated soil}} \times 100]$$

2.3 Inoculum level test

The growth substrate for culturing *S. rolfsii* inoculum was prepared by using rice husk rice grain substrate (RHRG) according to Muis & Quimio, (2006). Rice husk and rice grain (8:13, rice rusk: rice grain (w/w)) were thoroughly mixed and soaked in 2% (w/v) sucrose solution in 1hr and drained off. About 250 g mixture of substrate was placed in cellophane bags. They were autoclaved at 121ºC for 20 mins and cooled down for one day. The sterilized substrate was inoculated with a 5 mm diameter mycelial disc of *S. rolfsii* that was taken from the margin of actively growing cultures using a cork borer, and incubated at room temperature for 2 weeks. Five hundred grams of sterilized soil (the mixture of soil, sand and well-decomposed compost in a 2:1:2 ratio) was thoroughly mixed with eight inoculum levels of *S. rolfsii* (1.25g, 2.5g, 5g, 10g, 20g, 30g, 40g and $50g\,kg^{-1}$ soil) respectively and incubated for 2 days. Then, five surface sterilized tomato seeds were sown in *S. rolfsii* inoculated soil . Five surface sterilized tomato seeds were also sown in the uninoculated soil (0 % inoculum) for control and incubated for 14 days. The experiment was conducted in CRD with four replications. At 14 days after sowing, seedling stands in inoculated and uninoculated pots were recorded and mortality (%) was calculated as described in the pathogenicity test.

2.4 Reaction of tomato varieties to collar rot disease

Tomato varieties were collected from different areas (Table 1). *S. rolfsii* inoculum preparation and inoculation were prepared the same as described in the inoculum level test. The experiment was carried out in CRD with four replications. At 28 days after sowing, seedling stands in inoculated and uninoculated pots were recorded and seedling mortality (%) was calculated as described in the pathogenicity test. The host reaction was evaluated by using the standard rating scale (1-9) developed by ICRISAT (Nene , Haware, & Reddyet al., 1982) (Table 2).

التعليق **[DS21]:** It was preferable to test the pathogenicity of the isolates in plastic pots.

التعليق **[DS22]:** How many replicates per isolate?

Table 1. List of the test varieties of tomato

No.	Tomato Variety	Growth Type	Plant Age	Source
1	Paddamyar -1	Determinate	120	VFRDC
2	Paddamyarni	Determinate	120	VFRDC
3	Lora -25	Semi-determinate	110 - 120	Ayeyarwaddy Seed
4	Pan -111	Semi-determinate	110 - 120	Agro Bio Product
5	Red Diamond 115	Semi-determinate	110 - 120	East West Seed
6	VF-cherry	Indeterminate	-	VFRDC
7	Local (VF)	Indeterminate	-	VFRDC
8	Kyaukme Gaung Seine	Indeterminate	-	DAR
9	India	Indeterminate	-	DAR
10	Kyarchayyar (Htainkangyi)	Indeterminate	-	DAR

VFRDC = Vegetable, Fruit Research and Development Centre, Hlegu,Yangon

DAR= Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw

Table 2. Standard rating scale of resistance and susceptibility of plants developed by ICRISAT

Scale	Mortality (%)	Reaction
1	0	Resistant (R)
2-3	≤10	Moderately resistant (MR)
4-5	11-20	Tolerant (T)
6-7	21-50	Moderately susceptible (MS)
8-9	≥51	Susceptible (S)

2.5 Molecular Characterization of *Trichoderma* Isolates

The four *Trichoderma* isolates (YAU-Tri.sp1, YAU-Tri.sp2, YAU-Tri.sp3, and YAU-Tri.sp4) were provided by the Department of Plant Pathology, YAU, and one commercial *Trichoderma* inoculant was obtained from Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw. The total genomic DNA of *Trichoderma* isolates was extracted from mycelium using DNeasy$^{@}$ Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer instructions. The two sequence-characterized amplified region (SCAR) primer sets (Parmar et al., 2015) for *T. harzianum* and *T. viride* were used for identifying the *Trichoderma* spp. (Table 3). PCR reactions were conducted in the Swift^{TM} MaxPro Thermal Cycler (Esco Micro Pte Ltd, Singapore). Each 25µl reaction volume contains amplified products of 2µl of template DNA, 12.5µl of 2× Taq Plus PCR Master mix (CoWin Biotech Co., Ltd., China), 2µl of each forward and reverse primer, and 6.5 µl of ddH$_2$O. PCR amplification reactions were used for *T. harzianum* and *T. viride* with the program of preheating in 95°C for 5mins and followed by 30cycles of denaturing (95 °C for 30 secs), annealing (61°C and 55°C for 1min) and extension (72°C for 30secs) and final extension (72°C for 5 mins). Amplified DNA products were analyzed by electrophoresis in 1.5 % (w/v) agarose gel stained with ethidium bromide (5 µg ml^{-1}) in Tris-acetate-EDTA (TAE) buffer. 100bp ladder was used as a standard marker. DNA was visualized and photographed under UV light (CI-310B, Bio-CRAFT, Japan).

Table 3. Sequence of SCAR primers used for species identification

Primers	Primer sequences	Species	Annealing Temperature	PCR product size
HAR220FP5	5´-CTTTTGGTTTGACACGGTTCT-3´	*T. harzianum*	61°C	220 bp
HAR220RP5	5´-AAGCTTTGAAGTTGCGAGGA-3´			
VIRI900FP7	5´-TACGCTCCAGGCTACCACTT-3´	*T. viride*	55°C	900 bp
VIRI900RP7	5´-GAGATGAGCTCCTTGCTGCT-3´			

2.6 Effect of *Trichoderma* isolates against the mycelial growth of *S. rolfsii*

The inhibitory effect of four *Trichoderma* isolates from YAU and a commercial *Trichoderma* isolate from DAR were evaluated on *S. rolfsii* by dual culture technique as described by Dennis & Webster (1971). The experiment was laid out in CRD with five replications. Three days after incubation, the radial growth (mm) of the pathogen in the treated and control plates were measured. Percent growth inhibition was calculated with the following formula proposed by Vincent (1947).

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where,

      C = mycelial growth (mm) of a pathogen in the control plate

      T = mycelial growth (mm) of a pathogen in a treated plate

2.7 Effect of *Trichoderma* isolates on tomato plant growth and collar rot disease

The most susceptible variety, Kyaukme Gaung Seine resulted from the varietal reaction test, and the most virulent isolate, SR5 resulted from the pathogenicity test was used in this experiment. *S. rolfsii* and *Trichoderma* inoculum preparation and inoculation were prepared the same as described in the inoculum level test. Soil inoculation was carried out in plastic pots (15cm in diameter). The sterilized soil was thoroughly mixed with *S. rolfsii* inoculum at the rate of 30g kg^{-1} soil and each *Trichoderma* substrate at the rate of 35 g kg^{-1} soil (Nay Nay Oo, 2015). About 2 kg of the mixture was placed in each pot and incubated for 3 days. Tomato seeds were surface sterilized with 5% NaOCL for 5 mins, washed three times with sterilized water, and pregerminated for 1 day. Five surface sterilized seeds were sown in inoculated pots and also sown *S. rolfsii* only inoculated pots for control. The experiment was laid out in a CRD with six replications. In this experiment, there were six treatments, namely, YAU-Tri.sp1 + *S.rolfsii,* YAU-Tri.sp2 + *S. rolfsii,* YAU-Tri.sp3 + *S. rolfsii,* YAU-Tri.sp4 + *S. rolfsii,* DAR-Tri.sp + *S. rolfsii* and *S. rolfsii* only (positive control).

At 60 days after sowing, plant growth parameters per plant (plant height, fresh and dry weight), the number of diseased and healthy plants were recorded. Harvested tomato plants were oven-dried at 70°C for 72 hrs to determine the dry weight (Sani, Hasan, Uddain, & Subramaniam et al., 2020). Disease incidence percent (Cooke, 2006) and disease control percent (Pascual, Toda, Raymondo & Hyakumachib et al., 2000) were calculated using the following formulae.

$$\text{Disease incidence}(\%)= \frac{\text{No. of diseased plant}}{\text{Total no. of plant}} \times 100$$

$$\text{Disease control}(\%)= \frac{\text{Infected plants in untreated pot-Infected plants in treated pot}}{\text{Infected plants in untreated pot}} \times 100$$

2.8 Data analysis

The collected data were analyzed by Statistix software (version 8.0). Treatment means were compared by using the least significant difference (LSD) test at 0.05 level.

3. RESULTS AND DISCUSSION

3.1 Morphological characteristics of seven *S. rolfsii* isolates

A total seven *S. rolfsii* isolates were obtained from the collected diseased samples, and the isolates were named as SR1, SR2, SR3, SR4, SR5, SR6, and SR7. There were two types of colonies among the seven isolates. Among them, six isolates were fluffy and one isolate was a compact colony in the present study. The characteristics of hyphae were hyaline with thin cells and cross walls are sparse. The white mycelium was observed with narrow mycelial strands in the aerial mycelium and hyphal diameter ranged from 4.17 μm to 5.56 μm. All isolates produced cottony white mycelia. In all tested isolates, the main branch hyphae had clamp connections on each side of the septum. *S. rolfsii* mycelium of tomato isolate usually formed many narrow mycelial strands in the aerial mycelium and ranged from 4.0-9.8μm in width (Kwon and & Park, 2002). The growth rate ranged from 23.53 mm to 29.79 mm day^{-1}. The highest mycelial growth was observed in SR5 (29.79 mm day^{-1}) followed by SR4, SR1, SR3, SR2, SR6 and SR7. Daunde et al., (2020) also recorded that significantly maximum mycelial growth per day (31.45 mm) and minimum mycelial growth per day (21.62 mm) was formed in *S. rolfsii* isolate. The size of

sclerotia ranged from 0.99 mm to 1.15 mm and sclerotia in all tested isolates were spherical shape and brown colour. The size of sclerotia were measured 1.0 - 3.0 mm ,mostly spherical and brown colour (Kwon & Park, 2002). The largest sclerotia were produced by the SR1 isolate and its diameter was (1.15 mm) and the smallest one was produced by the SR5 isolate (0.99 mm). The maximum number of sclerotia (722 petridish[-1]) was observed in the SR5 isolate and the minimum number of sclerotia (203 petridish[-1] plate) was produced by the (SR6) isolate (Table 4).

Table 4. Morphological characteristics of *S. rolfsii* isolates

| *S. rolfsii* isolates | Mycelial character | | | Sclerotium character | | | Clamp connection |
	Colony texture	Growth rate (mm day^{-1}) [a]	Hyphal diameter (µm) [b]	No. of sclerotia plate[-1] [c]	Size (mm) [d]	Color	
SR1	Fluffy	28.67 b	5.56 a	374 c	1.15 a	Brown	Present
SR2	Fluffy	26.74 c	4.79 c	217 d	1.01 bc	Brown	Present
SR3	Fluffy	28.49 b	4.82 c	368 c	1.03 bc	Brown	Present
SR4	Fluffy	29.36 a	5.30 b	427 b	1.07 b	Brown	Present
SR5	Fluffy	29.79 a	4.71 c	722 a	0.99 c	Brown	Present
SR6	Fluffy	26.45 c	5.19 b	203 d	1.01 bc	Brown	Present
SR7	Compact	23.53 d	4.17 d	-	-	-	Present
LSD$_{(0.05)}$		0.66	0.21	51.74	0.07		
P		<0.001	<0.001	<0.001	<0.001		
CV%		1.84	8.53	10.29	13.69		

a mean values of 5 replications, b mean values of 30 observations,

c mean values of 4 observations, and d mean values of 30 observations

3.2 Pathogenicity and inoculum level test of *Sclerotium rolfsii*

All *Sclerotium rolfsii* isolates were pathogenic to test tomato variety,Kyaukmae Gaung Seine. The seedling mortality (%) of seven isolates (SR1, SR2, SR3, SR4, SR5 and SR6) were 100 % except SR7 isolate (53%). In the present study, SR5 isolate produced the maximum number of sclerotia and the highest mycelial growth rate among all tested isolates. Pandi et al., (2017) also reported that the most virulent isolates showed the highest mycelium growth rate and produced the maximum number of sclerotia in the culture. Therefore, the SR5 isolate was selected for further experiments.

In the next experiment of effective inoculum level of *S. rolfsii* in tomato collar rot disease inoculation, SR5 isolate was tested with eight inoculum levels. Inoculum level (30 g kg^{-1}) caused 85% mortality and 100% mortality were observed in 40 g and 50 g kg^{-1} inoculum levels. Another report was recorded by Zaghloul , Neweigy, Hanafy, & Khalifaet al. (2008) evaluated that soil was infested with different rates i.e. 1, 3, 5, 7 and 9% of soil weight for *S. rolfsii,* inoculum densities (3 and 5% kg[-1] soil) for EB-R1 and Sa-R strains recorded moderate percentage (68% - 80%) of pre and post-emergence damping-off of tomato. Therefore, it can be assumed that the effective inoculum level was 30 g kg^{-1} inoculum level for soil inoculation.

التعليق **[DS25]:** In this study, it is not possible to correlate the pathogenicity of an isolate with the number of sclerotia and growth rate as long as there are five isolates with the same pathogenicity (seedling mortality)

3.4 Reaction of tomato varieties to collar rot disease

Among the varieties, the seedling mortality percent of Paddamyar-1and Kyaukme Gaung Seine varieties were found to be highest at 90 % and it was statistically similar to that of Paddamyarni, VF cherry, India (85%), and Kyarchayyar variety (80%) respectively. Moderately and high mortality percent were found in Lora-25 (55%) and Pan-111(65%). The lowest seedling mortality percent, 50 % was observed in the Red Diamond 115 variety. Based on the seedling mortality percent, 2 reactions, namely, moderately susceptible (MS) and susceptible(S), were occurred among the ten tested varieties (Table 5). Most of the test varieties showed susceptible reactions. However, Red Diamond115 varieties exhibited moderately susceptible reactions.

Table 5. Seedling mortality percent and reaction of ten tomato varieties to collar rot disease at 28 days after sowing

| Variety	Seedling Mortality (%)*	Host Response
T_1 -Paddamyar-1	90.00 a	Susceptible(S)
T_2 – Paddamyarni	85.00 a	Susceptible(S)
T_3 - Lora -25	55.00 bc	Susceptible(S)
T_4 - Pan-111	65.00 b	Susceptible(S)
T_5- Red Diamond115	50.00 c	Moderately susceptible (MS)
T_6 - VF-cherry	85.00 a	Susceptible(S)
T_7 - Local (VF)	85.00 a	Susceptible(S)
T_8 - Kyaut Me Gaung seine	90.00 a	Susceptible(S)
T_9 – India	85.00 a	Susceptible(S)
T_{10} - Kyarchayyar (Htainkangyi)	80.00 a	Susceptible(S)
LSD $_{(0.05)}$	14.44	
P	< 0.001	
CV%	12.99	

*Means of four replications

means followed by the same letters in the same column are not significantly different at LSD 5% level

3.5 Molecular identification of *Trichoderma* isolates

Species-specific markers HAR 220FP5 and HAR220RP5 for T.richoderma harzianum and VIRI900FP7 and VIRI900RP7 for T.richoderma viride (Parmar et al., 2015) were used to identify five *Trichoderma* isolates. According to the result of PCR amplification in the former primer set, a sharp single band was found at 900 bp in each lane 1 to 4 which corresponds to the isolates YAU-Tri.sp1, YAU-Tri.sp2, YAU-Tri.sp3, and YAU-Tri.sp4, and these isolates can be identified as *T. viride*. In the latter primer set, a single and bright band was observed between 200 and 300 bp in lane 5, DAR-Tri.sp isolates. Thus, the commercial *Trichoderma* isolate from DAR was *T. harzianum*. According to the molecular characterization based on PCR with species-specific primers, the four *Trichoderma* isolates from YAU were *T. viride,* and the commercial isolate, DAR-Tri.sp was *T. harzianum*.
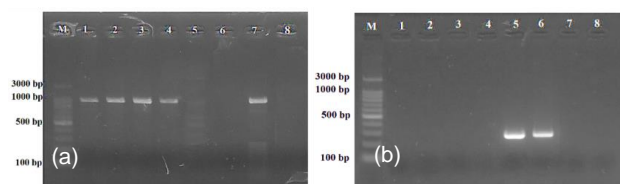
Plate 1 PCR amplification of genomic DNA of different fungal species using

> **(a) VIRI900FP7 and VIRI900RP7 primers, (b) HAR220FP5 and HAR220RP5 set, where lane M = 100 bp DNA ladder, 1 = YAU-Tri.sp1, 2 = YAU-Tri.sp2, 3 = YAU-Tri.sp3, 4 = YAU-Tri.sp4, 5 = DAR ,6= *T. harzianum* check, 7 = *T. viride* check and 8 = negative check (ddH₂O)**

3.6 Effect of *Trichoderma* isolates against the mycelial growth of *S. rolfsii*

YAU-Tri.sp4 was found to have the most suppressive effect on the mycelial growth of *S. rolfsii* isolate showing the highest inhibition percent (71.11%). Inhibition percent by YAU-Tri.sp1, YAU-Tri.sp2, YAU-Tri.sp3 and DAR-Tri.sp against *S. rolfsii* was 66.56 %, 70.22 %, 67.11 %, and 61.33%, respectively. Among them, DAR-Tri.sp showed the lowest inhibition percent on mycelial growth of the test pathogen than that of the other *Trichoderma* spp. Nay Nay Oo (2015) also found that *T. harzianum* showed 61.1% inhibition of mycelial growth of *S. rolfsii* from tomato isolate *in vitro*. Similarly, in the evaluation of four bioagents against *S. rolfsii* in tomato, *T. viride* caused maximum mycelial growth inhibition (72.67 %), and *T. harzianum* (57.57 %) (Banyal, Mankotia, & Sugha, 2008).
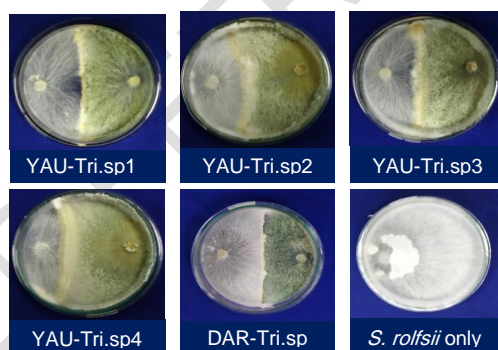
Plate 2. Inhibition effect of five *Trichoderma* isolates on the mycelial growth of *S. rolfsii* (SR5) at 4 days after incubation

3.7 Effect of *Trichoderma* isolates on tomato plant growth and collar rot disease

There were significant differences in plant height, fresh weight and dry weight of tomato plants among the tested *Trichoderma* isolates against collar rot disease compared to positive control (Table 6). At 60 days after sowing, the range of plant height was found to be 26.42 cm to 47.95 cm. The plant height of YAU -Tri.sp4, DAR-Tri.sp, and YAU -Tri.sp2 inoculated plants showed the highest plant height (47.95 cm), (47.82cm) and (47.28cm), respectively. Among them, the plant height of the YAU -Tri.sp4 inoculated plant (47.95cm) was significantly higher than that of the remaining treatments. The shortest plant (26.42 cm) was observed in *Trichoderma* untreated plant. Myo Zaw & Matsumoto (2020) also reported that the plant

height of one-month-old Japanese mustard spinach and tomato increased by 9.84 % and 7.00 % in *Trichoderma* treated home garden soil in comparison with non-treated control.

At 60 days after sowing, the highest fresh weight (7.89 g) was resulted in the individual plant inoculated(or treated) with DAR-Tri.sp. The lowest fresh weight (4.84 g) was observed in untreated plant. After 48 hrs of oven drying (72˚C), the dry weight of individual plants treated with YAU -Tri.sp4 and DAR-Tri.sp was higher than that of the plants treated with others. The highest dry weight (1.56 g) was obtained from YAU -Tri.sp4 and DAR-Tri.sp inoculated plants. The plant growth parameters were significantly higher than the positive control in all treatments .

The highest disease incidence (83.33%) was observed in only *S. rolfsii* inoculated plants (positive control) at 60 days after sowing. In comparison with positive check, the test variety treated with each *Trichoderma* isolate was found to reduce disease incidence ranging from 13.33 % to 23.33 % which caused percent disease control ranging from 71.89 % to 83.94 %. The disease incidence was not significantly different among the plants treated with each *Trichoderma* isolate. Out of five *Trichoderma* isolates, the lowest disease incidence and the highest percent disease control were found on the test variety treated with YAU-Tri.sp4. A similar observation was found that YAU-Tri.sp2 and YAU-Tri.sp4 significantly reduce the disease incidence of root and stem rot of soybean in field tests (Myo Zaw et al., 2024). In percent disease control, there were no significant differences among the *Trichoderma*-treated plants, ranging from 71.89 % to 83.94 %. The numerical highest percent disease control (83.94%) was observed in *T. viride*, YAU-Tri.sp4 treated plants.

Table 6. Effect of selected *Trichoderma* isolates on growth parameters and collar rot disease incidence of tomato plants inoculated with *S. rolfsii* at 60 days after sowing

Treatments	Plant height(cm)[x]		Fresh weight (g)[x]		Dry weight (g)[x]		Disease incidence (%)[x]		Disease control (%)[x]
T$_1$ - YAU-Tri.sp1	43.88	b	6.05	b	1.17	bc	23.00	b	71.89 a
T$_2$ - YAU-Tri.sp2	47.28	a	7.60	a	1.35	ab	16.66	b	79.92 a
T$_3$ - YAU-Tri.sp3	42.25	b	6.01	b	1.13	bc	23.33	b	71.89 a
T$_4$ -YAU-Tri.sp4	47.95	a	7.67	a	1.56	a	13.33	b	83.94 a
T$_5$ - DAR-Tri.sp	47.82	a	7.89	a	1.56	a	20.00	b	75.90 a

T_6 - Positive Control [y]	26.42 c	4.84 c	0.98 c	83.33 a	-
LSD$_{(0.05)}$	1.94	0.97	0.29	11.12	13.84
P	<0.001	<0.001	<0.001	<0.001	0.33
CV%	3.85	12.33	19.13	31.43	15.17

xMeans of five replications, Means followed by the same letters in the same column are not significantly different at LSD 5% level

4. CONCLUSION

Seven isolates of *S. rolfsii* were pathogenic to tomatoes, though there were differences in pathogenicity levels. Among them, SR5 isolate from Kanoo village was the most virulent resulting the highest mycelium growth rate and maximum sclerotia production. The effective inoculum level of *S. rolfsii,* SR5 isolate on RHRG substrate for tomato collar rot disease was 30 g kg^{-1} soil. In the varietal reaction study, all tested varieties were susceptible except Red Diamond115 varietie which showed moderately susceptible reaction. According to the molecular identification, four *Trichoderma* isolates from YAU were *T. viride* and commercial *Trichoderma* isolate from DAR was *T. harzianum.* Based on the results of the dual culture experiment, YAU-Tri.sp4 was the most effective isolate in inhibiting mycelial growth. Moreover, YAU-Tri.sp4 has plant growth promotion effect on tomato plants, followed by commercial *Trichoderma* isolate, DAR-Tri.sp, and all the tested *Trichoderma* isolates treated plants showed lower disease incidence than without *Trichoderma* treated one. Additionally, YAU-Tri.sp4 isolate showed the highest disease control ability against the collar rot of tomato. Therefore, it can be concluded that *T. viride* (YAU-Tri.sp4) could be used as the commercial biocontrol agent for controlling the tomato collar rot disease. However, further investigations of the effect of *T. viride* isolate (YAU-Tri.sp4) on crop growth and yield under different field conditions would be necessary before commercial applications.

REFERENCES

Adhikari, P., Shrestha, S. M., Manandhar, H. K., & Marahatta, S. (2022). Effect of *Trichoderma* isolates on *Sclerotium rolfsii* Sacc. *Journal of Agriculture and Forestry University*, 299–310.

Banyal, D. K., Mankotia, V., & Sugha, S. K. (2008). Integrated management of tomato collar rot caused by *Sclerotium rolfsii. Journal of Mycology and Plant Pathology*, *38*(2), 165–167.

Benítez, T., Rincón, A. M., Limón, M. C., & Codón, A. C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology, 7*(4), 249–260.

Brindhadevi, S., Thiruvudainambi, S., & Theradimani, L. (2021). In vitro evaluation of bio agents against *Sclerotium rolfsii* Sacc. causing southern blight of tomato. *The Pharma Innovation Journal*, *10*(3), 135–137.

Chowdary, G. B. S. M., Jameema, G., & Charishma, K. V. (2024). Collar and Stem Rot Pathogen-*Sclerotium Rolfsii*: A review. *Plant Archives*, *24*(1), 67–72.

Cooke, B. (2006). Disease assessment and yield loss. In D. S. Netherlands (Ed.), *The epidemiology of plant diseases* (pp. 43–80). Netherlands.

Daunde, A. T., Apet, K. T., Navgire, K. D., & Khandare, V. S. (2020). *Integrated Management of Collar Rot of Chilli Caused by Sclerotium rolfsii Sacc. International Journal of Current Microbiology and Applied Sciences*,*9*(6), 2187-2194.

Dennis, C., & Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society, 57*, 25–39.

FAOSTAT(2022). Statistics Division. Food and Agriculture Organization of the United Nations. Viale delle Terme di caracalle 00153 Rome, Italy (xxxx@fao.org).

Garcia-gonzalez, J., Hansen, M. A., Strawn, L. K., & Rideout, S. L. (2021). An Overview of Southern Blight , Caused by *Sclerotium rolfsii. Virginia Cooperative Extension*, 4.

Kataria, H., & Grover, R. (1976). Some factors affecting *Rhizoctonia solani* by systemic and non-systemic fungicides. *Annals of Applied Biology*, *82*, 267–278.

Kwon, J.-H., & Park, C.-S. (2002). Stem Rot of Tomato Caused by *Sclerotium rolfsii* in Korea . *Mycobiology*, *30*(4), 244.

Ministry of Agriculture, Livestock and Irrigation (MOALI). (2023). Myanmar Agriculture Sector in Brief. Myanmar Agricultural Service (MAS), Naypyitaw, Myanmar. P. 63.

Muis, A., & Quimio., A. J. (2006). Biological control of banded leaf and sheath blight disease (*Rhizoctonia Solani* Kuhn) in corn with formulated *Bacillus Subtilis* Br23. *Indonesian Journal of Agricultural Science*, 1–7.

Mullen, J. (2001). Southern blight, southern stem blight, white mold. *The Plant Health Instructor*, *10.*

Myo Zaw, M., & Matsumoto, M. (2020). Plant growth promotion of *Trichoderma virens*, Tv911 on some vegetables and its antagonistic effect on fusarium wilt of tomato. *Environmental Control in Biology*, 58(1), 7–14.

Myo Zaw, M., Htet wai wai kyaw, Yi Yi Mon, May Thu Tin, Aye Thet Hnin, Zin Mar Soe, Tin Aye Aye Naing (2024) Effect of on Natural Infestation of Fungal Diseases and Yield of Soybean (*Glycine max* L.) in Yezin, *Proceedings of the Centennial Research Conference of Yezin Agricultural University*, 198, 7-8.

Nay Nay Oo (2015). Variation in morphological characteristics of Sclerotium rolfsii and biocontrol efficacy of *Trichoderma harzianum* on this fungus, Yezin Agricultural University,Myanmar.

Nene, Y. L., Haware, M. P., & Reddy, M. O. V. (1982). Chickpea disease resistance screening techniques. *Bull. No. 10 ICRISAT, Hyderabad, India*. (p. 10).

Pandi, V. K., Gopalakrishnan, C., & Janahiraman, V. (2017). Cultural and Morphological Variability in *Sclerotium rolfsii* Causing Stemrot Disease. *International Journal of Current Microbiology and Applied Sciences*, *6*(6), 3090–3097.

Parmar, H. J., Hassan, M. M., Bodar, N. P., Lakhani, H. N., Umrania, V. V, & Golakiya, B. A. (2015). Development of SCAR marker for Specific Detection of *Trichoderma harzanium* and *Trichoderma viride. American Journal of Microbiological Research*, *3*(1), 45–49.

Pascual, C. B., Toda, T., Raymondo, A. D., & Hyakumachib., M. (2000). Characterization by conventional

منسّق:الفرنسية (فرنسا)

techniques and PCR of *Rhizoctonia solani* isolates. *Plant Pathology Journal*, *49*, 108–118.

Pavan Kumar.S, A. S. and K. R. B. (2018). Symptomology of major fungal diseases on tomato and its management. *Journal of Pharmacognosy and Phytochemistry*, *7*(6), 1817–1821.

Prasad, Vidya Sagar, B., Rao, G. U., & Koteswar, S. R. (2017). In vitro Evaluation of Fungicides and Biocontrol Agents Against Damping Off Disease Caused by *Sclerotium rolfsii* on Tomato. *International Journal of Pure & Applied Bioscience*, *5*(4), 1247–1257.

Sani, M. N. H., Hasan, M., Uddain, J., & Subramaniam, S. (2020). Impact of application of *Trichoderma* and biochar on growth, productivity and nutritional quality of tomato under reduced N-P-K fertilization. *Annals of Agricultural Sciences*, *65*(1), 107–115.

Szabo, K., Dulf, F. V., Teleky, B. E., Eleni, P., Boukouvalas, C., Krokida, M., Kapsalis, N. (2021). Evaluation of the bioactive compounds found in tomato seed oil and tomato peels influenced by industrial heat treatments. *Foods*, *10*(1), 1–16.

Vincent, J. M. (1947). Distribution of fungal hyphae in the presence of certain inhibitors. *Nature Reviews Microbiology*, *150*, 850.

Zaghloul, R. A., Neweigy, N. A., Hanafy, E. A., & Khalifa, N. A. (2008). Effectiveness of Bio-control Agents Against Tomato Soil Borne Pathogens.*Third Environment Conference, Faculty of Science, Zagazig University., 2008, 123- 142* (pp. 123–142).

التعليق [DS26]: