Compost Seed of *Trichoderma harzianum* UD12-102 in Controlling Collar and Stem Rot of Tomato Caused by *Sclerotium rolfsii*

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ABSTRACT

Antagonistic fungus, *Trichoderma harzianum* UD12-102, exhibited 90% inhibition against *Sclerotium rolfsii* in vitro and 80% survival of tomatoes infected by *S. rolfsii* in vivo. Moreover, the antagonistic fungi increased the effectiveness of a commercial fungicide (vitavax) in controlling *S. rolfsii* in tomatoes. In field experiments, composts were used as carriers for *T. harzianum* UD12-102 inoculum preparation. Following *S. rolfsii* inoculation, the survival percentages of tomato plants were not significantly different with all treatments receiving *T. harzianum* UD12-102 antagonist. However, the inoculum prepared with compost B (inoculated the antagonist at beginning of composting) resulted in a high survival percentage (more than 60%) with 4 weeks of infection, while the survival percentage of control plants dramatically decreased on week 2 (8.35%), and the plants died after 3 weeks due to *S. rolfsii*. The compost was a good alternative carrier for antagonistic fungi inoculation and was friendly with soil environments.

1. INTRODUCTION

Sclerotium rolfsii is one of the most destructive diseases of tomatoes (*Lycopersicon esculentum* Mill.), causing collar rot and stem rot. It is prevalent in all parts of the world, especially temperate regions. The mycelia of *S. rolfsii* expand rapidly in soil and attack the tissue of the tomato stem near the soil. Subsequently, the fungus develops abundant mycelia on the stem followed by yellowing and wilting of the leaves. Finally, the infected plant dies. This disease occurs in both greenhouse and field-grown tomatoes and results in losses of up to 30% of crop (Thiribhuvanamala et al., 1999). The fungus produces many sclerotia that can survive in soil and plant residue long term. Control of the pathogen is provided by chemical, biological and solarization. Presently, many chemicals can inhibit *S. rolfsii*, such as vitavax, calixin, benodanil, campogran M, busan, captan, brassical, carbendazim and mancozeb, etc. (Pan and Sen, 1977; Ganeshan, 1997; Akram et al., 2008). However, chemical control is an environmentally harmful method and should be strictly regulated. Fungicide causes huge problems for environments, damaging the health of organisms as farmers use them to control diseases in unregulated amounts. Thus, fungicides remain in high concentrations in soil affecting ecosystem balance by killing organisms such as insects and worms which are vital parts of the food chain. Moreover, accumulation of fungicide in soils can contaminate ground water, rivers, and lakes. This causes biological magnification which is toxic to aquatic life, animals and humans.

Biological control, the addition of antagonistic fungi to soil, is an interesting method for management of *Sclerotium rolfsii*. Several researchers have reported the inhibition of soil-borne pathogen by *Trichoderma* species such as *T. virens, T. harzianum, T. atroviride* and *T. asperellum*, etc. (Wijesinghe et al., 2010; Schubert et al., 2009). Okereke et al. (2007) reported the efficacy of *T. harzianum* and some plant extracts to control *S. rolfsii* in tomato seedlings. *T. harzianum* showed the highest inhibitory activity (80.3%).The objective of this study was the isolation of antagonistic strains against *S. rolfsii* causing collar and stem rot in tomato plants in Northeast Thailand. The hypothesis was that local strains would be more effective to inhibit the strain of *S. rolfsii* found in local areas. The effect of antagonistic strains integrated with fungicide on plant disease control was investigated with the aim of reducing agricultural chemical use. Compost seed was selected for use in

antagonistic inoculum preparation. The seed inoculums of *T. harzianum* UD12-102 were studied by application in compost for use as biocontrol and the efficiency of seed inoculum types was observed to control collar and stem rot under field conditions.

2. METHODOLOGY

2.1 Isolation of fungi from soil

Soil samples were collected from the tomato growing area by sampling 5 - 10 cm below the soil surface from a plot in the northeastern region of Thailand. Ten grams of soil was diluted in 90 mL of sterile water with shaking for 30 min. The dilutions at 10^{-2} and 10^{-3} were spread on a Rose Bengal agar with 30 µg/mL of streptomycin. The plates were next incubated at 30 ± 2 ºC for 24-72 h. Different colonies of fungi were selected and cultivated on potato dextrose agar (PDA) and incubated at 30 ± 2 ºC for 7 days. The cultures were then stored at 4 ºC on PDA slant for further study.

2.2 Plant pathogen

Active pathogen, *Sclerotium rolfsii*, was provided by Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University.

2.3 Screening of antagonistic fungi

The selected fungi were evaluated for antagonistic activity against *S. rolfsii* by dual culture method. The mycelial disc (0.4 mm in diameter) of 3 day-old *S. rolfsii* was placed on the edge of PDA agar plate (90 mm of plate diameter). Meanwhile, the mycelial disc of selected 7 day-old fungi was placed on the opposite side. The inoculated plates were incubated at 30 ± 2 ºC for 3-7 days. The PDA plate with only *S. rolfsii* served as the control. Antagonistic activity was determined by measuring pathogen mycelial growth distance from the original point of the mycelial disc to hyphal tip compared with the control plate. The percentage inhibition was calculated by the following equation: Inhibition Percentage (%) = (A1 - A2) /A1 x 100, where A1 was radial growth of *S. rolfsii* in the control, and A2 was radial growth of *S. rolfsii* in the dual culture (Asran-Amal et al., 2010).

2.4 Determination of antagonistic fungi against *Sclerotium rolfsii* in greenhouse

The seed inoculums of antagonistic fungi were prepared. Sorghum grains were used as a substrate for fungal growth; the method was modified from Dongarmart et al. (2015). The sorghums grains were immersed in tap water for 24 h. After immersion, 200 g of sorghum was placed in a plastic bag and covered with cotton cork. The bags were autoclaved for 15 min at 121 ºC. Each autoclavable plastic bag was inoculated with 10 mycelial discs (8 mm in diameter) of 3 day-old antagonistic fungus. They were then incubated at 30 ± 2 ºC for 7 days, and used for seed inoculums. In parallel, the seed inoculums of *S. rolfsii* were prepared in the same way as seed inoculums of antagonistic fungi. For tomato seeds (Sida variety), the surface was washed in 70% ethyl alcohol for 1 min and rinsed with sterile water for 5 min each, 5 times. After that, the seeds were shaken in 0.5% sodium hypochlorite for 10 min followed by 5 times in sterile water for 5 min each. The surface sterile seeds were planted in a plastic tray containing sterile peat moss for 3 weeks to produce a tomato seedling. Groups of four seedlings were transferred into pots containing 1 kg of sterile materials (40% sand, 50% vertisol soil and 10% peat). After 7 days, 50 g of each antagonistic inoculum were added around the collar of the tomato seedlings. After 7 days, the seedlings were inoculated with 50 g of pathogenic inoculum at the same point in the pot. In the greenhouse test, 18 treatments were designed (Table 1) using commercial fungicide (Vitavax) at 500 mg/mL as a control, and with antagonistic fungi for the experiment. The vitavax was applied to plants at the same time as inoculation of pathogen inoculums. The tomato plants were watered daily under greenhouse temperatures and disease severity was determined 3 weeks after planting. The rate of damage of each replication caused by *S. rolfsii* disease was judged on the following scale (El-Mohamedy et al., 2014): 0 = no infection; 1 (1-20 % of plant damage) = no internal browning, root lesions at the points of emergence of lateral roots; 2 (21-40 % of plant damage) = brown tap root with slight internal browning at the tip of the tap root; 3 (41-60 % of plant damage) = moderate internal browning of the entire tap root; 4 (61-80 % of plant damage) = severe internal browning extending from tap root into lower stem above soil surface, abundant lesions on distal roots and 5 (81-100 % of plant damage) = dead plant. The percentage of plant survival was calculated from plant damage.

Table 1. Method of testing antagonistic fungi (UD4-8, UD14-4, UD12-10, UD12-102 and KL10-12) against *Sclerotium rolfsii* in greenhouse with or without commercial fungicide

Treatment	Method
T1	Tomato seedlings inoculated with UD4-8
T2	Tomato seedlings inoculated with UD4-8 + *S. rolfsii*
T3	Tomato seedlings inoculated with UD4-8 + *S. rolfsii* + Vitavax
T4	Tomato seedlings inoculated with UD14-4
T5	Tomato seedlings inoculated with UD14-4 + *S. rolfsii*
T6	Tomato seedlings inoculated with UD14-4 + S. *rolfsii* + Vitavax
T7	Tomato seedlings inoculated with UD12-10
T8	Tomato seedlings inoculated with UD12-10 + *S. rolfsii*
T9	Tomato seedlings inoculated with UD12-10 + *S. rolfsii*+ Vitavax
T10	Tomato seedlings inoculated with UD12-102
T11	Tomato seedlings inoculated with UD12-102 + *S. rolfsii*
T12	Tomato seedlings inoculated with UD12-102 + *S. rolfsii* + Vitavax
T13	Tomato seedlings inoculated with KL10-12
T14	Tomato seedlings inoculated with KL10-12+ *S. rolfsii*
T15	Tomato seedlings inoculated with KL10-12 + *S. rolfsii*+ Vitavax
T16	Tomato seedlings +Water
T17	Tomato seedlings inoculated with *S. rolfsii*
T18	Tomato seedlings inoculated with *S. rolfsii*+ Vitavax

2.5 Field experiments

The experiment was determined in field conditions, using a post-harvest rice field (Khon Kaen, Thailand) The antagonistic fungus was cultivated on sorghum for seed inoculums, as mention previously. Compost used as a fungal inoculum carrier consisted of dried rain tree (*Samanea saman*) leaves mixed with cow manure (ratio 1:1) plus 10% fine rice bran, 10% molasses, 10% soybean meal and 5% urea. The composting was carried out in a 50 kg porous bag for ventilation, turned once a week and kept at 60-80% moisture for 90 days. For compost A, 10% of the fungal seed inoculums were added before using the compost for 7 days while compost B had 10% of the fungal seed inoculums added at the beginning of composting. For plot preparation, rice stubble was cut and ploughed before planting for 2 weeks, after which soil was ploughed in regular furrows. The plot was lifted 30 cm in height from the watercourse; the size was 1 x 5 m. Planting holes were dug in 20 cm radius and 10 cm deep in a zigzag arrangement with each hole 50 cm apart. Tomato seeds (Sida) were soaked in water for 1 h and planted in soil for 30 days. The seedling was transferred into the plot, two tomato seedlings per hole. After 7 days of planting, the antagonistic fungus inoculums were applied around tomato plants. Seven days later, the pathogenic inoculums were added around the collar of plant. The antagonistic fungal seed inoculums in compost A and compost B were tested for reduction of *S. rolfsii* disease by application of 30, 50 or 70 g of compost and comparison with controls. Therefore, 12 treatments were conducted. The disease severity and percentage of plant survival was analyzed every week for 4 weeks; both fresh and dry weight of the plants were recorded on week 4 after pathogenic inoculation.

2.6 Identification of antagonistic fungi

The isolate performing with highest efficiency against *S. rolfsii* in the greenhouse experiment was identified. The morphological characteristics were determined according to Harman and Kubicek (2002). The molecular study was analyzed by Mahidol University-Osaka University Collaborative

Research Center for Bioscience and Biotechnology (MUOU:CRC) using internal transcribed spacer (ITS) rDNA, ITS1 and ITS4 primers. The sequences were aligned and identified using BLAST program of NCBI database.

2.7 Statistical analysis

The greenhouse and field experiments utilized completely randomized design (CRD) with five replications and thirty replications, respectively. The data were analyzed using standard analysis of variance (ANOVA) with the MSTAT-C program. Multiple comparisons of means were made by using Duncan's multiple range test (DMRT).

3. RESULTS

3.1 Antagonistic activity of isolated fungal strains

Five hundred and one fungal strains were isolated from soil samples and their antagonistic activity determined based on dual culture technique. Thirteen isolates showed strong activity to inhibit *S. rolfsii*, with percentages of inhibition above 40%. The antagonistic fungal mycelia grew rapidly and were found to exhibit greater growth than the pathogenic fungus. On other hand, the end of colony of *S. rolfsii* was covered by antagonistic fungi; dead brown mycelium was found in an inhibition region. Meanwhile, mycelium of *S. rofsii* was dramatically inhibited by five isolates of antagonistic fungi (UD4-8, UD14-4, UD12-10, UD12-102 and KL10-12) with percentages of inhibition from 80 to 90%.

3.2 Efficiency of 5 antagonistic fungi against *Sclerotium rolfsii* in greenhouse

Five isolates (UD4-8, UD14-4, UD12-10, UD12-102 and KL10-12) which obtained high inhibition activity from dual culture test were determined for suppression induction of tomato stem rot disease caused by *S. rolfsii* in pots. Figure 1 shows that the fungal isolate UD12-102 (T11) showed the highest survival percentage of tomato (80%) compared with control in T17 (Figure 2). Moreover, this isolate was able to reduce the disease or inhibit *S. rolfsii* infection better than commercial fungicide vitavax (T18). Another isolate UD12-10 (T8) had high potential to suppress disease. These 2 isolates indicated higher inhibition to *S. rolfsii* than the other 3 isolates according to in vitro and greenhouse tests. The combination of antagonistic fungi and fungicide revealed synergistic effects on antifungal activity by reducing plant disease from *S. rolfsii*

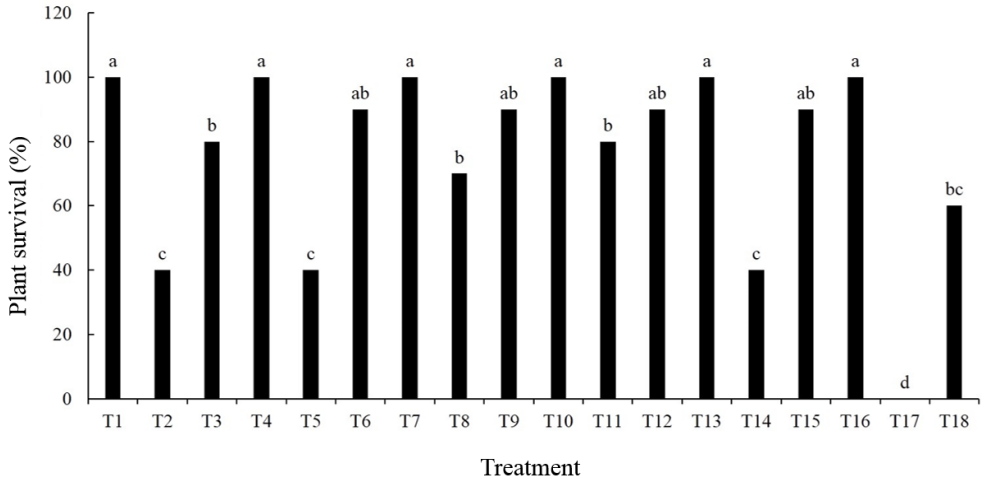

Figure 1. Effect of antagonistic fungi against *Sclerotium rolfsii* under greenhouse conditions. Treatments (T1-T18) were shown in Table 1 and the disease severity was determined in week 3 of planting. Identical letters indicated treatments which were not significantly different (P = 0.05) according to Turkey test.

3.3 Effect of the antagonistic fungus on tomato against *Sclerotium rolfsii* in field

The antagonistic fungus, UD12-102, was prepared in sorghum as seed inoculums and in carriers as compost inoculums to investigate effective inoculums against plant pathogen in the field. Table 2 shows the percentage of plant survival using UD12-102 in different carriers against *S. rolfsii*. The first week after the pathogenic inoculation, treatment with sorghum seeds and

compost A (30 g and 50 g), and compost B (50 g) showed more than 80% plant survival with significant values compared with control. Plant survival rapidly decreased in the second week with compost A treatment, while the control with *S. rolfsii* showed a dramatic decrease. Two treatments, sorghum seed and compost B at 50 g, had the highest inhibition with 73.35% of plant survival in the third week. By the fourth week, the 50 g compost B exhibited the highest plant survival. However, the sorghum seeds and compost B indicated more effective inoculums than compost A, even though all treatments had no significant difference in plant survival. When plant growth was observed (Table 3), plant weight was not significantly different in all treatments including controls without *S. rolfsii* inoculation. The tomato plants showed symptoms of *S. rolfsii* infection (Figure 3) in all treatments except T10 and T11. The sorghum seeds and compost B demonstrated the efficiency of seed preparation for using UD12-102 in reduction of plant disease, but not in promotion of plant growth.

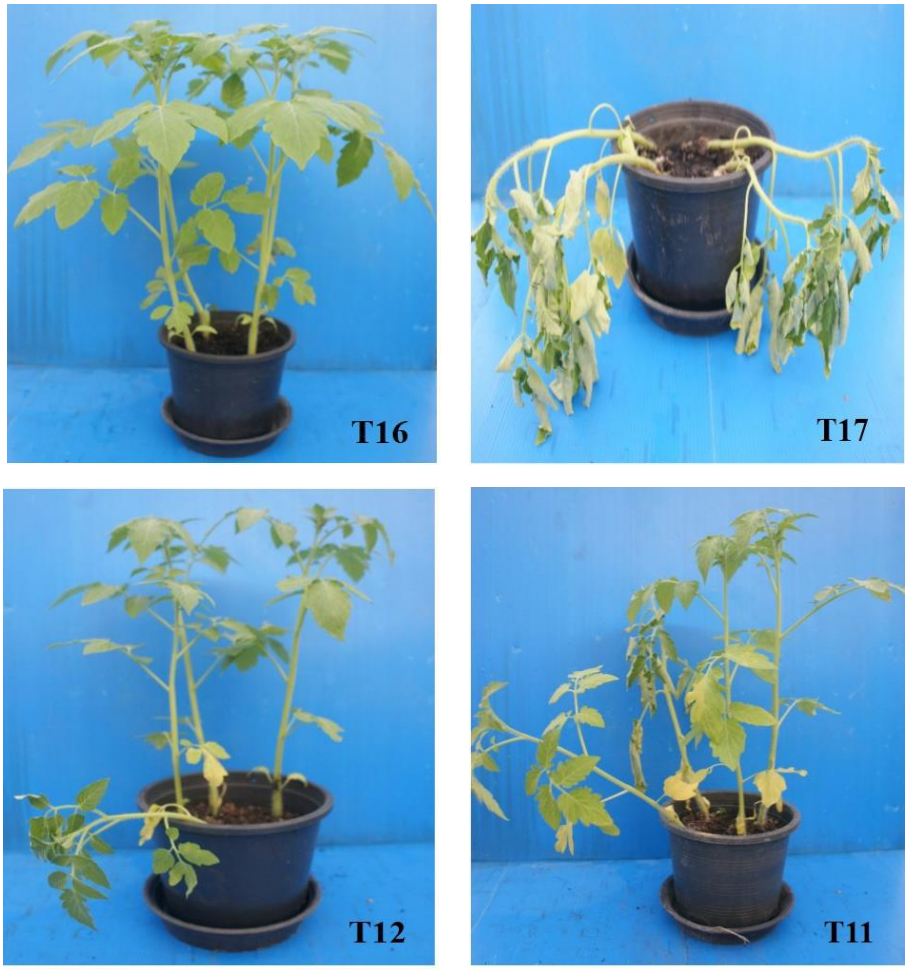

Figure 2. Tomato plants were grown in greenhouse conditions under treatment without *S. rolfsii* (T16); with *S. rolfsii* (T17); with UD12-102, *S. rolfsii* and vitavax (T12); with UD12-102 and *S. rolfsii* (T11) show wilted symptoms after 7 days inoculation of pathogen.

Table 2. Survival of tomato plants after inoculation with UD12-102 in different seed carriers against plant disease caused by *Sclerotium rolfsii* under field conditions.

Treatment		Plant survival (%)			
		Week 1	Week 2	Week 3	Week 4
T1	30 g sorghum seed + *S. rolfsii*	85.00^{ab}	73.35^{ab}	61.65^{bc}	58.35^{abc}
T2	50 g sorghum seed + *S. rolfsii*	83.35^{ab}	73.35^{ab}	73.35^{ab}	65.00^{ab}

Table 2. Survival of tomato plants after inoculation with UD12-102 in different seed carriers against plant disease caused by *Sclerotium rolfsii* under field conditions. (cont.)

Treatment		Plant survival (%)			
		Week 1	Week 2	Week 3	Week 4
T3	70 g sorghum seed + *S. rolfsii*	78.35^{ab}	70.00^{ab}	70.00^{abc}	66.65^{ab}
T4	30 g compost A + *S. rolfsii*	83.35^{ab}	65.00^{bc}	65.00^{bc}	45.00^{bc}
T5	50 g compost A + *S. rolfsii*	83.35^{ab}	61.65^{bc}	53.35^{cd}	48.35^{bc}
T6	70 g compost A + *S. rolfsii*	73.35^{bc}	65.00^{bc}	58.35^{c}	48.35^{bc}
T7	30 g compost B + *S. rolfsii*	78.35^{ab}	70.00^{ab}	65.00^{bc}	65.00^{ab}
T8	50 g compost B + *S. rolfsii*	81.65^{ab}	81.65^{ab}	73.35^{ab}	68.35^{ab}
T9	70 g compost B + *S. rolfsii*	71.65^{bc}	71.65^{ab}	65.00^{bc}	63.35^{ab}
T10	Without inoculation	100^{a}	100^{a}	100^{a}	100^{a}
T11	50 g sorghum seed	100^{a}	100^{a}	100^{a}	100^{a}
T12	50 g *S. rolfsii* seed	48.35^{c}	8.35^{c}	0^{e}	0^{c}

Identical letters in each column indicated that treatments were not significantly different at $p < 0.05$

Table 3. Tomato plant weight after inoculation with UD12-102 in different seed carriers against plant disease caused by *Sclerotium rolfsii* under field conditions at week 4.

Treatment		Fresh weight (g)	Dry weight (g)	Fresh/dry weight
T1	30 g sorghum seed + *S. rolfsii*	52.56^{e}	11.61^{cd}	4.52^{abc}
T2	50 g sorghum seed + *S. rolfsii*	61.75^{cd}	13.09^{cd}	4.71^{abc}
T3	70 g sorghum seed + *S. rolfsii*	59.26^{de}	12.78^{cd}	4.63^{abc}
T4	30 g compost A + *S. rolfsii*	42.17^{f}	9.54^{e}	4.42^{bc}
T5	50 g compost A + *S. rolfsii*	41.19^{f}	9.34^{e}	4.39^{c}
T6	70 g compost A + *S. rolfsii*	37.79^{f}	8.66^{e}	4.35^{c}
T7	30 g compost B + *S. rolfsii*	66.35^{cd}	14.19^{c}	4.67^{abc}
T8	50 g compost B + *S. rolfsii*	68.03^{c}	13.96^{c}	4.89^{a}
T9	70 g compost B + *S. rolfsii*	66.97^{c}	14.29^{c}	4.69^{abc}
T10	Without inoculation	82.91^{b}	17.26^{b}	4.81^{a}
T11	50 g sorghum seed	99.21^{a}	20.73^{a}	4.78^{ab}
T12	50 g *S. rolfsii* seed	0^{g}	0^{f}	0^{d}

Identical letters in each column indicated that treatments were not significantly different at $p < 0.05$

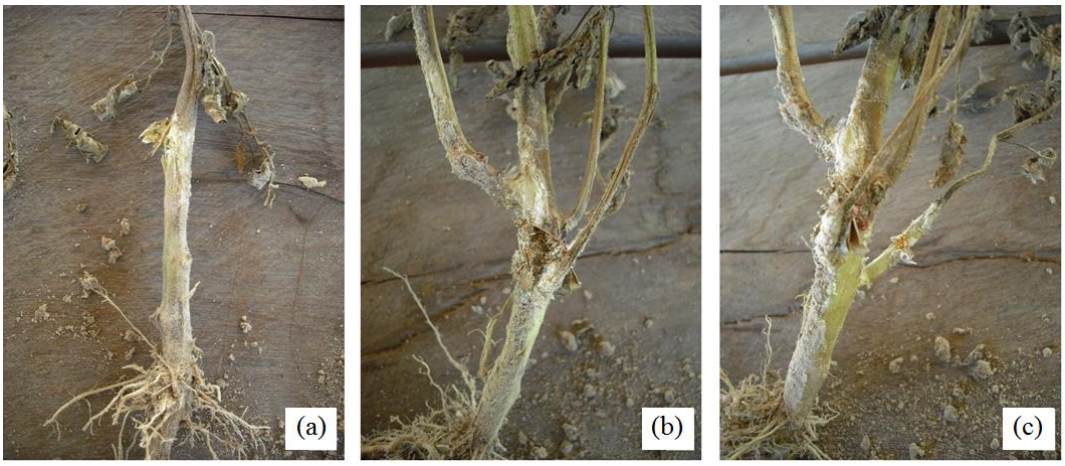

Figure 3. Symptoms occurring in tomato plants infected by *S. rolfsii* for 4 weeks; (a) dark-brown lesions on the stem and wilting of the leaves; (b,c) severe infected tissue with white and fluffy mycelium.

3.4 Identification of UD12-102 antagonistic fungus

The isolate UD12-102 showed the colony on PDA with white mycelia and green spores. Under microscopy, it presented septate hyphea, branch conidiophores, flask shape phialides and globose green spores (Figure 4). This strain was identified as *Trichoderma harzianum* with 100% similarity using ITS rDNA.

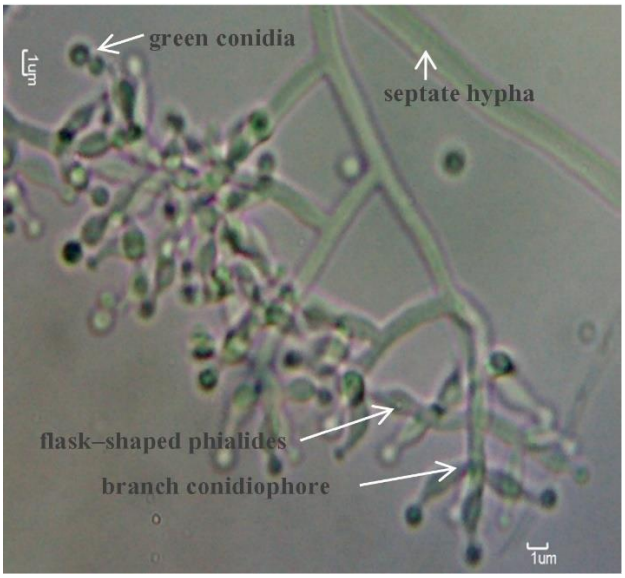

Figure 4. Morphology of *T. harzianum* UD12-102 under 400X microscopy which presented septate hyphea, branch conidiophores, flask shape phialides and globose green conidia as indicated by arrow.

4. DISCUSSION

Trichoderma spp. is well known as an antagonist species since it can grow rapidly and produce bioactive compounds against fungal pathogens of plants. Different strains exhibit different properties based on habitat (host) or environment. Therefore, *T. harzianum* UD12-102 was isolated from tomato field for inoculum developing against collar and stem rot caused by *S. rolfsii*. Ganesan et al. (2007) reported the effect of *T. harzianum* (ITCC-4572) to control *S. rolfsii* causing stem rot in peanut, (*Arachis hypogaea* L.) showing the percentage of inhibition at 60.5. Meanwhile, Jomduang and Sariah (1997) studied the ability of antagonistic fungi to inhibit *S. rolfsii* by dual culture technique, and found that the percentages of inhibition of *T. harzianum* and *Gliocladium viren* were 64.44 and 70.48, respectively. Both reports correlate to our findings for *T. harzianum* UD12-102, which showed high efficiency in controlling *S. rolfsii* mycelium growth with a percentage of inhibition at 90%. Moreover, the highest survival percentage of tomato plant under greenhouse experiment was performed by *T. harzianum* UD12-102 (80%), related to the result of dual culture test. This figure was similar to previous research; Okereke et al. (2007) studied *T. harzianum* against *S. rolfsii* on tomato seedlings and found that *T. harzianum* showed 80.3% of inhibition. An explanation for this inhibition could be that *Trichoderma* grew around the tomato root and used exudates of root produced by *S. rolfsii* infection as food to propagate and colonize in rhizosphere or in root tissue (Fahima and Henis, 1990). The chemical fungicide protecting seed and seedling of plants from *S. rolfsii* are widespread and highly efficient. Vitavax (carboxin) was used in this study. This agent was the most effective to control *Corticium rolfsii* in cowpea seedling (Rahmanet al., 1994) and the best against rust (Patil and Patil, 2010), but was less effective against *S. rolfsii* in tomato (Kulkarni, 1980). Our study showed vitavax giving 60% survival of tomato seedling infected with *S. rolfsii*, which was more effective than some antagonists (UD4-8, UD14-4 and KL10-12). Interestingly, the isolate UD12-10 and UD12-102 inhibited disease better than vitavax and also proved to be more effective in cooperation with vitavax. The reason for this may be synergistic effects between vitavax and bioactive compounds of the antagonistic fungi. The combination of carboxin with thiram showed high inhibition against *S. rolfsii* in in vitro tests (Manu et al., 2012). The results confirmed that vitavax or carboxin work well with other fungicides or biocontrol, resulting in complete inhibition against *S. rolfsii* in tomato seedling. However, the concentrations of vitavax should be considered when used with antagonistic fungi; a reduction in the amount of vitavax when combined with antagonistic fungi would decrease chemical contamination in soil, and mean less toxicity to the antagonistic fungi. This may decrease the harmful effects of chemicals on the ecosystem whilst boosting the efficiency of fungal disease control.

The antagonists applied in field work are usually produced in seed inoculum formulation, either plant seed coat formulation or seed inoculum with carriers. Agricultural waste is a useful food base as it is friendly to environment, cheap or of no cost, easily prepared and enriched with organic

matter, meaning antagonists do not require a high amount of inoculums. In this study, dried rain tree leaves, cow manure, fine rice bran, molasses and soybean meal from industry and agriculture were fermented and used as carriers of the antagonist. In field experiments, survival percentages of tomato plant 4 weeks after *S. rolfsii* inoculation indicated that the sorghum seeds of *T. harzianum* UD12-102 controlled plant disease, whilst seeds from compost B obtained *T. harzianum* UD12-102 at the beginning for 3 months composting. Compost A obtained *T. harzianum* UD12-102 for 7 days before inoculation into tomato plant. It showed lower survival percentage, but performed with no significant differences. As expected, the compost materials were used as substrates by antagonistic fungi. *Trichoderma* spp. could degrade cellulotic biomass by producing cellulases as hydrolytic activity; this enzyme system is also involved in plant root interaction and induction of systematic resistance (Saravanakumar et al., 2016) including *T. harzianum* that could produce β-glucanase and cellulase (Thrane et al., 1997; Libardi et al., 2017). It is possible that *T. harzianum* UD12-102 might produce cellulolytic enzyme, thus, the fungi in the compost B might remain and increase within the compost, resulting in strong antagonist characteristics. Danon et al. (2010), in their study of the amount of fungi within compost by using denaturing gradient gel electrophoresis (DGGE) technique, detected antagonistic fungi (*Trichoderma, Chaetomium, Geomyces* and *Penicillium*) suppressed *S. rolfsii*. In addition, Zmora-Nahum et al. (2008) reported that *Sclerotium* was totally inhibited by compost extract. Compost A showed low effectiveness, possibly caused by short composting (1 week) of the antagonistic fungal, giving low quantity of antagonistic compounds. The antagonistic fungi was able to use substrates in compost B for growth and secondary metabolite production including antifungal agents. The optimum amount of the compost B was 50 g of inoculation. However, no significance was shown between the compost A and B. Therefore, composts are an alternative carrier for inoculum seed applied in field conditions. The weight of surviving plants was measured to confirm efficiency of the antagonist; the values of fresh and dry weight of all treatments were related to survival percentage of plant, and proved similar to positive controls without *S. rolfsii*. These results implied that *T. harzianum* UD12-102 was able to control collar rot and stem rot on tomato plants caused by *S. rolfsii*, but could not promote tomato growth. This is in contrast to *T. koningii*, which demonstrated effective prevention of *S. rolfsii* while also promoting tomato growth (Tsahouridou and Thanassoulopoulos, 2002).

5. CONCLUSIONS

Trichoderma harzianum UD12-102 is an antagonistic fungus against plant pathogen *Sclerotium rolfsii*. Used alone, it prevented disease caused by *S. rolfsii*. However, *T. harzianum* UD12-102 also showed positive results working in combination with vitavax by increasing inhibition of pathogen growth compared with only vitavax treatment. The compost utilized was a good carrier for cultivation of the antagonistic fungus and applying seed inoculums in field. *T. harzianum* UD12-102 in the 50 g compost B proved the best treatment to control disease after one week infection, and over a four week period resulting in 82, 82, 73 and 68% of plant survival in each week, respectively. As for plant growth promotion, the ratio of fresh and dry weight of tomato plant was highest in the compost B treatment.

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