

## Biological Control of Sheath Diseases of Rice Caused by *Rhizoctonia oryzae* and *Rhizoctonia solani* by *Trichoderma* spp.

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The objective of this study, aims to select the effective antagonistic fungi to control sheath diseases of rice. The experiments were conducted in the laboratory and green house. In the laboratory, the antagonist fungi, *Trichoderma hazianum* T9, T18 and T13 *T. asperellum* and *T. koningii* isolate 67 were tested on their efficiency against *R. solani* and *R. oryzae* in dual cultures. The result showed that the isolates T9, T13, T18, 67 were able to inhibit the colony of *R. oryzae* and *R. solani*. The percent of colony inhibition was highly efficient against *R. oryzae* isolates VHN, VHN1-3 and SMN1-7 as 88%, 63% and 63%, respectively. For *R. solani*, isolates VNN, SMC1-2 and SMN1-5 exhibited the percent of inhibition as 41%, 40% and 36%, respectively. In the green house test, the result revealed the 3 effective isolates, T13, T18, 67 for control *R. oryzae*. The best method for application of isolates T18 and T13 were foliar spray, by which disease suppression was 92% and 87%, respectively. Isolate 67 was applied by root dip method and causing disease suppression of 87%. For *R. solani*, the isolate T18 was applied by soil treatment and expressed disease suppression of 61%. The best method for application of isolates T13 and 67 were the combination of soil treatment, root dip and foliar spray, by which disease suppression was 60% and 50%, respectively. All 3 isolates were significant difference ( $P < 0.05$ ) in disease suppression compared to control. This study suggests the biological control approach for sheath diseases of rice in Lao PDR.

**Keywords:** Sheath diseases, *Rhizoctonia solani*, *Rhizoctonia oryzae*, *Trichoderma* spp, biological control.

Rice is a staple food of Asia and part of the Pacific. All people in the world's consume rice for 85% of total production compared with 72% for wheat and 19% for maize. Sheath disease was a soil-borne disease<sup>1</sup> and caused by *R. solani* and *R. oryzae*. Sheath blight is one of most important diseases caused damage to rice crop in various regions of the world<sup>2</sup>. It has a wide host range and distributes in some field crops, such as corn, rice,

lawn grass and cucumber<sup>3</sup>. All cultivars of rice were susceptible<sup>4</sup>, but the degree of susceptibility is varied, yield loss ranged from 25-50%. Including in Brazil, rice was affected to economic loss as high as 32% in grain weight<sup>5</sup>. The susceptible cultivars, high yielding with large numbers of tillers, were greatly to the rapid increase in the incidence and severity of this disease<sup>6</sup>. Further-more, environmental conditions such as low light, cloudy days, high temperature and high relative humidity also favor to the disease. The pathogen survives for long periods and over several seasons as

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sclerotia and mycelia in the soil or in crop debris. These constituents play a role in the primary inoculum<sup>7</sup>. Different methods have been used to control sheath diseases. The most used methods are cultural practices, soil solarization, chemical and biological control. Application of fungicides, which have drastic effects on the soil biota, pollutes the atmosphere, and are environmentally harmful. Some potentially effective fungicides are highly phytotoxic to rice and, if the disease is not severe, these fungicides may reduce yield<sup>8</sup>. It is difficult to achieve control through fungicides, therefore, biological control may be effective in minimizing the incidence of sheath disease<sup>2</sup> and this method is not harmful to farmers, consumers and the environment. Biological control through the use of antagonistic fungus such as *Trichoderma* is a viable disease management. These are also potential agents in suppressing rice sheath diseases. The main modes of action of the biocontrol agent include competition for nutrients, competition for space production of cell wall degrading enzymes, production of antifungal diffusible and volatile metabolites and mycoparasitism. *Trichoderma* spp. are considered to be antagonistic to many plant pathogenic fungi including *Rhizoctonia solani*, *Sclerotinia* spp. and *Fusarium* spp.<sup>9</sup>

In Lao PDR, there are limited information for the application of biological control of sheath diseases. Therefore, in this study, the objective is to select the effective antagonist fungi to control sheath diseases of rice caused by *Rhizoctonia oryzae* and *Rhizoctonia solani* from Lao PDR. The information will be useful to the farmer for application biocontrol to reduce chemical use in the future.

## MATERIALS AND METHODS

### Fungal pathogen and antagonistic isolates

Isolates of *Trichoderma* spp. were obtained from the Plant Pathology Section, Plant Science and Agricultural Resource Department, Faculty of Agriculture, Khon Kaen University Thailand.

The sheath disease pathogens, *Rhizoctonia oryzae* and *Rhizoctonia solani* were isolated from rice fields in Vientiane capital and Sayabouly province of Lao PDR.

### In vitro assays

Test of the efficiency of antagonistic fungi *Trichoderma* spp. on *Rhizoctonia* in laboratory.

All antagonistic isolates were tested in a dual culture assay against *Rhizoctonia oryzae* and *Rhizoctonia solani* on PDA. Agar plugs of *Rhizoctonia* spp. were cut out by using cork borer (5mm diameter) at the edge of actively growing colonies of the pathogens and *Trichoderma* isolates, and were placed opposite each 1.5 cm from the edge of 9 cm diameter Petri dishes containing PDA. Petri dishes inoculated with *R. solani* or *R. oryzae* alone served as control. The experiment was replicated 4 times. The dual cultures were incubated at temperature 25 °C for 5 days<sup>7</sup>. The diameter of *R. solani* colony was measured (mm) and calculated percent of inhibition over control by the following formula given<sup>10</sup>. Percent inhibition over control =  $[(C-T)/C] \times 100$ ; where, C= radial mycelium growth, T = radial mycelium growth of fungus in treatment.

### Test of interaction between *Trichoderma* spp. and *Rhizoctonia* spp

Mycelia of *Trichoderma* spp. and *Rhizoctonia* were inoculated an opposite on the sterile 2% water agar disc laid on a sterile glass slide, the cover slip was placed over the agar disc and incubated at 28°C for 5 days. When the hyphae of *Trichoderma* isolates met the hyphae of the pathogen were inspected under a light microscope for coiling structures of *Trichoderma* hyphae on the hyphae of *R. solani*<sup>11</sup>.

### Green house test

The efficacy of the 3 *Trichoderma* isolates, *Trichoderma asperellum* (T13, T18) and *Trichoderma koningii* (67) was evaluated by the *in vitro* screening tests for their control of sheath disease of rice *in vivo* under green house condition.

### Seedlings preparation

Twenty-day-old rice seedlings Thadokham 8 variety, two seedlings per pot were transplanted in plastic pots of 30 cm diameter x 25 cm height, each filled with 2.5 kg of rice field soil). Pots were arranged 25 cm spaced out on benches. Seedlings were fertilized by NPK applied to the soil (15:15:15, 1g/pot) and watered regularly to keep them submerged. Nitrogen fertilizer was apply in the pots (46-0-0 1g/pot). In green house, temperatures were 28 - 34°C and 60 - 70% relative humidity.

**Antagonistic fungus preparation**

Active mycelia of *Trichoderma* isolates on PDA were cut in small pieces (5mm diameter). Mycelial plugs were put in sterilized 100g sorghum grains in a plastic bag and incubated for 14 days. The concentration of spores was determined with an Improved Neubauer haemocytometer (Precicolor, HBG, Giessen, Germany) and adjusted to 10<sup>7</sup> conidia/ mL.

**Pathogen inoculation**

The rice plants were inoculated with *Rhizoctonia* spp. at the maximum tillering stage by placing a 5 cm mycelial plug of *Rhizoctonia* spp. between the junction of the basal leaf sheath and the stem above the water line and wrapped with parafilm<sup>12</sup>.

**Experimental design**

The pots were arranged in a completely randomized design (CRD) with three replications and eight treatments.

**Application antagonist for control *Rhizoctonia* spp**

The antagonistic fungus was applied by following treatments.

Treatment 1. Soil treatment (ST): the *Trichoderma* spp. grown on sorghum grains were inoculated in soil at 10g/pot and incubated seven days before transplanting rice plants in pots.

Treatment 2. Root dip (RD): 21-day-old rice seedlings were uprooted and dipped in spore suspension in water of *Trichoderma* isolates (10<sup>7</sup> conidia/ mL) for 30 minutes and then transplanted in pots.

Treatment 3. Foliar spray (FS): rice plants were evenly sprayed with 10 ml (10<sup>7</sup> conidia/ mL)

of spore suspensions of *Trichoderma*, using a hand sprayer, at 3 stages: 24 h after inoculation of the pathogen, 7 days after the first spray and third after 15 days after first spray.

Treatment 4. Soil treatment and root dip: First, soil treatment (10g/pot) were incubated in the pot seven days, then seedling was dipped in *Trichoderma* spp. suspension (10<sup>7</sup> conidia/ mL) for 30 minutes and grown in the pot.

Treatment 5. Soil treatment and foliar spray: First, soil treatment (10g/pot) were incubated in the pot seven days, then foliar spray as Treatment 3.

Treatment 6. Root dip and foliar spray: as Treatment 2 mixed with treatment 3

Treatment 7. Soil treatment mixed with root dip and foliar spray: as treatment 1, treatment 2 and treatment 3 were mixed.

Treatment 8. Control: Plants inoculated with *R. solani* alone.

Rice plants of treatment 1-7 were inoculated with *R. solani* by method of inoculation described previously<sup>5</sup>.

**Observation and recorded the data**

The first observation on disease severity and infected tillers were recorded one-day before second spray of the biocontrol agent, and the second recorded after 15 days of third spray. Disease suppression in the treated and control plants were calculated using the following formulae<sup>13</sup>. Percent of Sheath blight suppression = [(C-T)/C] x 100; where, C= % Sheath blight suppression of control, T = % sheath blight suppression of treated sample.

**Table 1.** Hyphal distance of *Trichoderma* spp. and *Rhizoctonia* spp. in dual culture

<i>Rhizoctonia</i> spp.	Antagonist hyphal distance (mm)			
	T9 ( <i>T. harzianum</i> )	T13 ( <i>T. asperellum</i> )	T18( <i>T. asperellum</i> )	67 ( <i>T. konigii</i> )
VHN ( <i>R. oryzae</i> )	16 d	15 c	13 d	15 c
VHN1-3 ( <i>R. oryzae</i> )	40 c	44 b	33 c	38.3 b
SMN1-7 ( <i>R. oryzae</i> )	40.6 c	47 b	32.6 c	38.3 b
SMN1-5 ( <i>R. solani</i> )	56.6 a	59 a	57.3 ab	55.6 a
SMC1-2 ( <i>R. solani</i> )	53 ab	61.6 a	53.6 ab	55.6 a
VNN ( <i>R. solani</i> )	49 b	57 a	52.3 b	56 a
F-test	**	**	**	**
C.V.(%)	8.83	9.31	6.82	9.31

\*\* Significant difference at P <0.01 by LSD

Means followed by the same letter (s) in a column are not significantly different (P <0.05 by LSD).

**Table 2.** Control of *R. oryzae* (VHN) in green house experiment by suspension of *Trichoderma* T13 and T18 (*Trichoderma asperellum*) and 67 (*Trichoderma koningii*) for 14 days after inoculation

Treatment	Isolates											
	T13				T18				67			
	Plant height (cm)	Lesion area (mm <sup>2</sup> )	No. of infected tiller	Disease Suppression (%)	Plant height (cm)	Lesion area (mm <sup>2</sup> )	No. of infected tiller	Disease Suppression (%)	Plant height (cm)	Lesion area (mm <sup>2</sup> )	No. of infected tiller	Disease Suppression (%)
ST	97.16 a	36 bc	2.33	81.34	89.67 b	23 b	1.60	88	90 cd	32 b	2	83
ST+RD	92.5 c	53 bc	2	72	89 c	53 b	1.60	72	105.67 a	27 b	2	86
RD	94.33 bc	42 bc	2	78	89 b	20 b	1.60	89	92 cd	24 b	1.66	87
FS	96.33 ab	25 c	2	87	95 a	15 b	2.30	92	98 b	33 b	1.66	82
RD+FS	93 c	39 bc	1	79	95 a	57 b	2.60	70	95.66 bc	38 b	2	80
ST+FS	98 a	43 bc	1	77	95 a	48 b	2.30	75	95.66 bc	40 b	1.66	79
ST+RD+FS	95.66 ab	68 b	1	64	84 d	61 b	2.30	68	93.33 bcd	37 b	1.66	80
Control	89 d	193 a	2.33	89 d	193 a	2.60	89 d	193 a	2.33			
F-test	**	**	NS	**	**	NS	*	**	NS			
C.V.(%)	1.47	38.26	21.77	0.22	55.20	19.58	3.52	44.62	20.56			

\*\* Significant difference at P <0.01 by LSD, NS = non significant difference

\* Significant difference at P <0.05 by LSD

Means followed by the same letter (s) in a column are not significantly different (P <0.05 by LSD).

The disease suppression was calculated as follow: % sheath blight suppression = [(% sheath blight suppression of control - % sheath blight suppression of treated sample) / % sheath blight suppression of control] x100.

ST = soil treatment, RD= root dip, FS = foliar spray, RD+FS = root dip + foliar spray, ST+FS = soil treatment + foliar spray, ST+RD+FS = soil treatment + root dip + foliar spray

**Table 3.** Control of *R. solani* (SMN1-5) in green house experiment by spore suspension of *Trichoderma asperellum* T13 and T18 and *Trichoderma koningii* 67 for 14 days after inoculation

Treatment	Isolates												
	T13						T18						67
	Plant height (cm)	Lesion area (mm <sup>2</sup> )	No. of infected tiller	Disease Suppression (%)	Plant height (cm)	Lesion area (mm <sup>2</sup> )	No. of infected tiller	Disease Suppression (%)	Plant height (cm)	Lesion area (mm <sup>2</sup> )	No. of infected tiller	Disease Suppression (%)	
ST	51.33	120 ab	4.33 bcd	35	44.33	67 bc	3.66 b	61	44.33	67 bc	3.66 b	49.33	106 ab
RD	47.33	106 b	6.6 ab	42	48.33	100 b	4.33 b	42	48.33	100 b	4.33 b	49.33	113 ab
ST+RD	45	93 b	6 abc	50	43.33	135 ab	5.66 ab	39	43.33	135 ab	5.66 ab	52.66	111 ab
FS	49.66	80 b	3.66 cd	53	48	69 bc	4.33 b	59	48	69 bc	4.33 b	48	126 ab
ST+FS	49.66	120 ab	5.33 bcd	42	45.66	78 bc	3.66 b	57	45.66	78 bc	3.66 b	45	78 bc
RD+FS	41.66	96 b	4.33 bcd	48	48.33	100 b	4.66 b	46	48.33	100 b	4.66 b	40	100 ab
ST+RD+FS	43	70 bc	3 d	60	46.66	100 b	5.66 ab	46	46.66	100 b	5.66 ab	50	96 b
Control	48.33	186 a	8 a		48.33	186 a	186 a		48.33	186 a	186 a	48	186 a
Healthy	49.33	0 c	0 e		49.33	0 c	0 c		49.33	0 c	0 c	49	0 c
F-test	NS	**	**		NS	*	**		NS	*	**	NS	*
C.V.(%)	9	46.38	21.77		6.07	49.73	21.77		6.07	49.73	21.77	8.92	48.54

\*\* Significant difference at P <0.01 by LSD, NS = non significant difference

\* Significant difference at P <0.05 by LSD

Means followed by the same letter (s) in a column are not significantly different (P <0.05 by LSD).

The disease suppression was calculated as follow: % sheath blight suppression = [(% sheath blight suppression of control - % sheath blight suppression of treated sample)/% sheath blight suppression of control] x100.

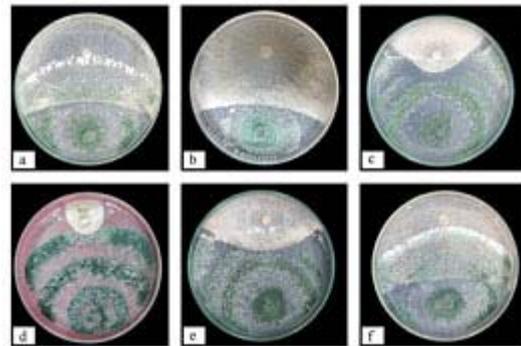
ST = soil treatment, RD= root dip, FS = foliar spray, RD+FS = root dip + foliar spray, ST+FS = soil treatment + foliar spray, ST+RD+FS = soil treatment + root dip + foliar spray.

**RESULTS**

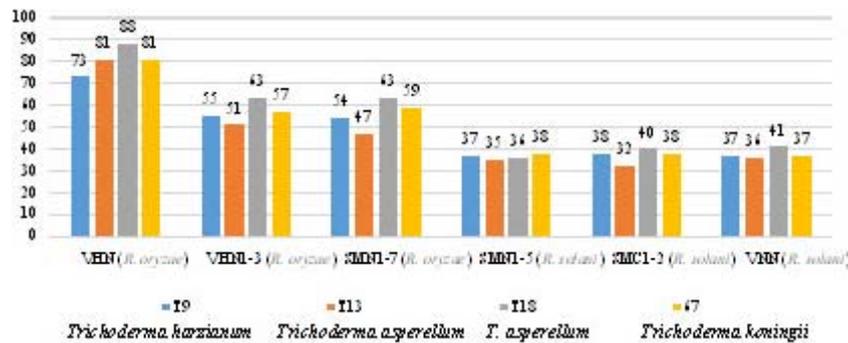
**Efficiency of *Trichoderma* spp. on *Rhizoctonia* spp**

All 4 isolates of *Trichoderma* spp. were effective against *Rhizoctonia* with significant difference from each other at P<0.05 (Table 1). The percent inhibition of *Trichoderma* spp. against *Rhizoctonia oryzae* (isolates VHN, VHN1-3 and SMN1-7) were higher than against *Rhizoctonia solani* (SMN1-5, SMC1-2 and VNN). *Trichoderma harzianum* T9 grew against hypha of *R. oryzae* isolate VHN was 16 mm of hypha distance. For *R. solani* isolate VNN, hypha distance was 49 mm, *Trichoderma asperellum* T13 and *Trichoderma koningii* isolate 67 grew against hypha of *R. oryzae* isolate VHN was 15 mm distance, but isolates of *R. solani* grew against hypha was not significant difference. *Trichoderma asperellum* T18 showed hypha against hypha of *R. oryzae* isolate VHN (13 mm distance). The percent of colony inhibition was high values to *R. oryzae* isolate VHN, VHN1-3 and SMN1-7 as 88%, 63% and 63%, respectively (Fig. 2). Furthermore, microscopic observation on

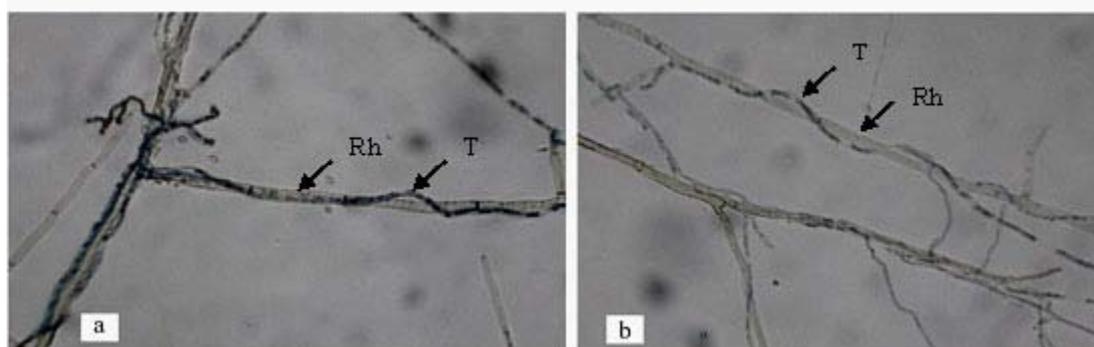
interaction of *R. oryzae* and *Trichoderma* resulted that mycelia of *Rhizoctonia oryzae* were thick when compared with only *R. oryzae*. Characteristic of *Rhizoctonia oryzae* mycelia could not grow over to *Trichoderma* spp. (Fig. 1). In contrast, *R. solani* isolates VNN, SMC1-2 and SMN1-5, the percents



**Fig. 1.** Dual culture of T18 with each isolate of *Rhizoctonia* on PDA within five day a) *T. asperellum* (T18) and SMC1-2 b) *T. asperellum* (T18) and SMN1-5 c) *T. asperellum* (T18) and SMN1-7 d) *T. asperellum* (T18) and VHN e) *T. asperellum* (T18) and VHN1-3 f) *T. asperellum* (T18) and VNN



**Fig. 2.** Percent inhibition of *Trichoderma* spp. against *Rhizoctonia oryzae* and *R. solani*



**Fig. 3.** Hypha interaction *Trichoderma* spp. and *Rhizoctonia solani* a) and b) *Trichoderma* spp. coiling hypha (T) around hypha of *R. solani* SMN1-5 (Rh)

of inhibition were 41%, 40% and 36%, respectively. (Fig. 2).

#### Test of interaction between *Trichoderma* spp. and *Rhizoctonia* spp.

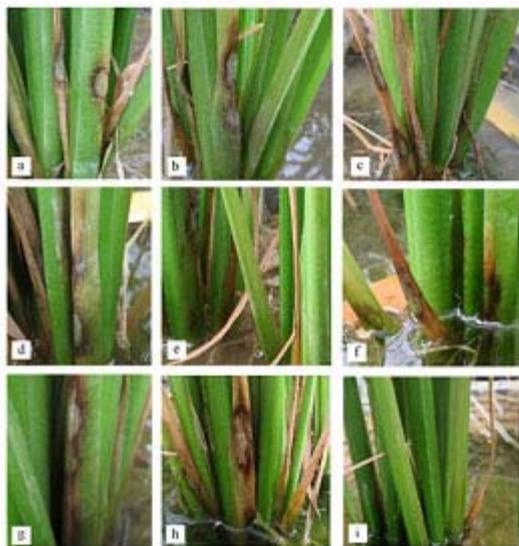
Microscopic inspection of the slide cultures between *Trichoderma asperellum* isolates T13 and *Rhizoctonia solani* isolate SMN1-5, it was clearly that *Trichoderma* isolates established the close-contact with pathogen by using coiling hypha around mycelia of *R. solani* as a result pathogen hyphae was ruptured and shrunk in size than the normal hyphae (Fig. 3).

#### Green house experiment

Test of biological control sheath disease using *Trichoderma* spp.

#### *Trichoderma* spp. against *Rhizoctonia oryzae* isolate VHN

The result in green house test was shown in Table 2, using 3 isolates of *Trichoderma* spp., which selected from laboratory experiment to test their efficiency for control *R. oryzae* in green house experiment. For isolate T13, the best method for application this isolate was foliar spray, which reflected the lesion area of 25 mm<sup>2</sup> compared to control treatment with highly significant difference



**Fig. 4.** Different applications of *Trichoderma* spp. for controlling *Rhizoctonia oryzae* isolate VHN in the green house experiment. a) Soil treatment b) Soil treatment + root dip c) Root dip d) foliar spray e) Root dip + foliar spray f) Soil treatment + root dip g) Soil treatment + root dip + foliar spray h) disease control i) healthy control.

( $P < 0.05$ ). The number of infected tiller was average 2 lesions compared to control treatment. However, it was not significantly different. The lesions were oval and ellipsoidal shape, gray color in the center and surrounded with dark brown color. Spraying T13 stimulated plant growth with the plant height value of 96.33 cm, which was of maximum compared to control treatment with statistically significant difference ( $P < 0.05$ ). Disease suppression was 87% (Table 2). Similarly, isolate T18 applied by spraying was the best method to control sheath spot with lesion area 15 mm<sup>2</sup>, number of infected tiller average 2.3 lesions disease suppression 92% and plant height 95 cm. Plant height and lesion area by using T18 were significantly different compared to control treatment ( $P < 0.05$ ). Symptom was greenish gray in the center, at dark brown margin (Fig. 4). On the other hand, isolate 67 was effective when used as root dip showing lesion area of 24 mm<sup>2</sup>, number of tiller infected average 1.66 lesions, disease suppression of 87%, plant height 92 cm. Using *Trichoderma* spp. isolates were effective for controlling sheath spot based on parameters recorded better than control treatment.

#### Test of biological control *Rhizoctonia solani* isolate SMN1-5 using *Trichoderma* spp.

The result in green house test was shown in Table 3 using 3 isolates of *Trichoderma* spp. which selected from laboratory experiment to test their efficiency for control *R. solani* in green house experiment. For isolate T13, the best method for application of this isolate was the combination between soil treatment, root dip and foliar spray, which reflected the lesion area of 70 mm<sup>2</sup>, compared to control treatment with highly significant difference ( $P < 0.05$ ). The number of infected tiller was average 3 lesions compared to control treatment with highly significant difference. The lesions were elongation to irregular shape, gray in the center and surrounded with dark brown color. Combination of T13 gave the plant height 43 cm, but not significant different compared to control treatment ( $P < 0.05$ ). Disease suppression was 60% (Table 3). However, isolate T18 with soil treatment was the best method to control sheath blight with lesion area 67 mm<sup>2</sup>, number of infected tiller average 3.66 lesions disease suppression 61% and plant height 51.33 cm. Lesion area by using T18 was significantly different to control treatment. However, plant height was not significant

difference compared to control treatment ( $P < 0.05$ ). Symptoms were green gray in the center, dark brown margin. In addition, isolate 67 was similar to isolate T13 effective when used as combination between soil treatment, root dip and foliar spray showing lesion area of 96 mm<sup>2</sup>, number of infected tiller average 4 lesions, disease suppression of 50%, plant height 50 cm. Using *Trichoderma* spp. isolates were effective for controlling sheath blight based on parameters recorded better than control treatment.

### DISCUSSION

In this study, antagonist isolates (T9, T13, T18, 67) were able to inhibit and overgrow the pathogenic fungi *R. oryzae* more high percents than *R. solani*. The antagonistic fungi *Trichoderma* spp. have been proved as an effective and selective enough against most of fungal plant disease. *Trichoderma* spp. have evolved numerous mechanisms that are involved in attacking other fungi. These mechanisms include competition for space and nutrients, mycoparasitism, production of inhibitory compound, inactivation of pathogen's enzymes<sup>14</sup>. According to result of screening strains of *Trichoderma viride* and *T. harzianum*, that inhibited/overgrew *R. solani* which the result were shown that some strains of antagonist induced well-defined inhibition zones between the advancing frontiers of their mycelial growth and that of the pathogen inhibiting the latter's growth, covering more than three-fourths of the surface<sup>15</sup>. However, some strains of antagonists uninhibited mycelium diameter of *R. solani*. Including, the fungal antagonists tested, which were active against the pathogen in soil (*R. solani* that isolated from potato). The result in *T. viride* was able to suppress mycelia growth under different conditions (at 20 °C and 12 °C)<sup>1</sup>. In addition, competition of antagonism and the experiment includes 13 treatments (12 *Trichoderma* spp. isolates) and one control with *R. solani* only. The result revealed all 13 *Trichoderma* spp. isolates evaluated were significantly different from the control in relation to antagonist to *R. solani*<sup>5</sup>. According to *Trichoderma harzianum* strains T22 and T39 produced azaphilone and butenolide, the secondary metabolites that shows markedly *in vitro* inhibition of *Rhizoctonia solani*, *Pythium*

*ultimum* and *Gaeumannomyces graminis* var. *tritici*<sup>16</sup>. In addition, *Trichoderma virens* produced gliotoxin toxic to both *R. solani* and *Sclerotinia americana*<sup>17</sup>.

Microscopic inspection in the slide cultures clearly showed that *Trichoderma* isolates established close contact with pathogen by coiling around the mycelia of *R. solani* as a result pathogen hyphae ruptured and shrunk in size than the normal hyphae. Based on the coils of antagonistic fungus were usually very dense and appeared to tightly encircle the hyphae of the pathogen. It usually formed appressorium-like structures or hook-shaped contact branches<sup>7</sup>. For the typical sign collapse of *R. solani* and loss of turgor, break down of the cell wall and hyphal disintegration were also occasionally seen. Coagulation and granulation of *R. solani* cytoplasm indicated the lysis. On the hyperparasitization of *R. solani* by *Trichoderma* spp. and stated that coiling, parallel growth, penetration of host hyphae<sup>18</sup>. In addition, coil formation is a common form of mycoparasitism and leads to the death of the parasitized fungus<sup>11</sup>. Parasitism is a mechanism present study between *Trichoderma asperellum* T13 and *Rhizoctonia solani* isolate SMN1-5. This showed evidently that *Trichoderma* spp. tested are potentially able to control both *R. solani* and *R. oryzae*.

In principle, the antagonistic fungus, *Trichoderma* spp., is able to control various plant disease, especially the soil-borne disease. It affects plant pathogen with different mechanisms such as competition, antibiosis and parasitism. It is possible that the plants produced salicylic acid, jasmonic acid or other substances, phytoalexin or proteinase inhibitor, which suppressed plant pathogens<sup>19</sup>. According, this study was shown the result in antagonist T13 and T18 isolates, the best method was foliar spray, which suppression of *R. oryzae* 87% and 92%, respectively. On the other hand, isolate 67 was effective when applied by root dip method showing disease suppression 87%. Moreover, 3 isolates of antagonists able to suppress *R. solani* only 53%, 61%, 50%, respectively. Spore suspension of *T. harzianum* sprayed on the leaves significantly reduced sheath blight severity and was more effective than soil treatment or seedling root dip<sup>6,20</sup>. Based on green house and laboratory tests, it was demonstrated that the application of the same four isolates of

*Trichoderma* to rice plants, grown under greenhouse condition, resulted in increased biomass, root length, and plant size, and reduced the severity of sheath blight<sup>6</sup>. Among the known mechanisms involved in achieving these results *Trichoderma* spp. can enhance growth of rice plant. Various species of fungi have been reported to produce auxins, which are key hormones effecting plant growth and development that can be produced by fungi in symbiotic interactions with plants<sup>21</sup>. Results from this present study exhibited that *Trichoderma* spp. tested can be used to control hyphal growth both *R. solani* and *R. oryzae* with plant growth, enhancement property.

### CONCLUSION

Test of the efficiency of antagonistic fungi *Trichoderma* spp. on *Rhizoctonia* in laboratory were highly significant difference. The percent of inhibition obtained from *Trichoderma* against *Rhizoctonia oryzae* (isolates VHN, VHN1-3 and SMN1-7) were higher than *Rhizoctonia solani* (SMN1-5, SMC1-2 and VNN). Test of interaction between *Trichoderma* spp. and *Rhizoctonia* spp., it was clearly that *Trichoderma* isolates established the close-contact with pathogen by using coiling hypha around mycelia of *R. solani* as a result pathogen hyphae was ruptured and shrunk in size than the normal. In green house, all 3 isolates of *Trichoderma* spp. were effective to control sheath disease.

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