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In-vitro screening *Trichoderma*spp. isolated from Sibolangit Conservation forest North Sumatera-Indonesia againstwhite-rot macromycetes

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ABSTRACT

In-vitro screening of Trichoderma spp. against five species white-rot macromycetes was conducted. All fungal species were isolated from Sibolangit Conservation Forest, North Sumatera-Indonesia. Trichoderma spp. were screened and isolated from soil samples by dilution method followed by pour plate method.Macromycetes were isolated from fresh fruiting body. By dual culture technique, antagonism both of the fungi were measured based on colony growth and percentage inhibition between species both of the fungi and mixed culture two species Trichoderma and one species white-rot. Results showed that 5 species of Trichoderma spp. (TR01, TR03, TR04, TR05,TR06) and white-rot (Lepiotanaucina, Ganodermaapplanatum, Trametesversicolor, Coltriciacinnamomea, and Phaelusschweinitzii) were isolated. Antagonism between one species Trichoderma and white-rot indicates all Trichoderma spp. have varying ability to inhibit mycelia growth of the white-rot,and mean inhibition percentage of each Trichoderma against macromycetes is higher than that of mixed cultures. IsolateTR01, TR03 and TR05 have potential to inhibit the white-rot.Whereas, mixed cultures(TR03+TR05) showed the highest inhibition against L. naucina and G. applanatum. Among the white-rot, Phaelusweinitziiis the most resistant to Trichodermaand the lowest inhibition percentagenot only between Trichoderma species but in mixed cultures as well.

Key words: *Trichoderma*, Macromycetes, Antagonism, Percentage inhibition

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INTRODUCTION

Sibolangit Conservation Forest is tropical rain forest that has area 24.85 ha, located in Deli Serdang County, North Sumatera Province, Indonesia. Altitude 550 m above sea level with average temperature 23.4^{o}C. Rainfall 3000-4000 mm annually. Geographically located between 98^{o} 36’ 36’’ and 98^{o} 36’ 56’’ E and 03^{o}17’50’’ – 03^{o}18’39’’ N. Vegetation of the forest is dominated by hardwood broad leave trees such as *Dalbergia latifolia*, *Pterocarpusindicus*, *Shorea*, *Ficus*spp.*Samaneasaman*, *Terminalia*sp. *etc.* Forest ecosystem provides habitat for many organisns. Wood-rotting macromycetes have essential role on nutrient cycle for other organisms especially carbon and nitrogen[1, 2]. However, most trees are non-durable and subject to attack by a wide range of the fungi[3]. Many species of wood decaying macromycetes are facultative parasite and cause damage on living hardwood in forest[4]. Forest close to plantation such as rubber tree, palm oil tree, cacao etc. become inoculum source for facultative parasitic macromycetes.The use of bio-control agents to protect microorganisms as a natural enemy could be used to control colonization parasitic of other microorganisms. The basic concept is to employ the natural balance ecological antagonisms of selected organisms against specific target. Increase concern over the environmental effects of chemical biocidesto control the growth of parasitic macromycetes.

Several species of *Trichoderma* showed competitive activity against wood decay fungi[4].Highley and Ricard[5] demonstrated the ability of *Trichoderma* to inhibit the growth of brown and white-rot fungi. The effectiveness of *Trichodermavirens* to protect wood from white and brown-rot fungi was studied by Highley 1997[6]. Rapid

colonization *Trichoderma* spp. and production of lytic enzymes inhibit the growth of pathogenic fungi[7, 8, 9]. Species of wood decay fungi showed different sensitivity when challenged by *Trichoderma*[10]. High enzymatic activity of *T. viride* and *T. harzianum* to inhibit plant pathogen fungi was reported by Kumar*et al*. 2012 [11]. The purpose of our work was to determine the ability of *Trichoderma* spp. isolated from Sibolangit Conservation Forest in North Sumatera-Indonesiaagainst five species white-rot macromycetes(*Lepiotanaucina, Ganodermaapplanatum, Trametesversicolor, Coltriciacinnamomea*, and *Phaelusschweinitzii*) under laboratory condition.

MATERIALS AND METHODS

Soil samples and isolation of*Trichoderma* spp.:The cultures of all fungal isolates used in the present study were isolated from Sibolangit ConservationForest, North Sumatera-Indonesia.About 200 g soil samples for fungal isolation were collected from 5sampling sites according to Rabeendran*et al*.[12]. Each site was replicate 3 times. Soil was taken at 10 cm depth, sample were sieve and air dried for 3-5 d at 18^{o}C. After drying, the soil were kept at 10^{o}C until used. Five-fold serial dilutions of each soil samples were prepared in sterilize distilled water and 1 mL of diluted sample was poured on the petri dishes (90 mm) contain liquid Potato Dextrose Agar (PDA) medium. Plates were incubated at 29^{o}C for 5 days. Colonies of *Trichoderma* spp. were isolated in fresh PDA medium and observed morphologically. The purified isolates were identified according to Gams and Bissett2002 [13] then stored in refrigerator and used during course of study.

Culture tissue of white-rot macromycetes: Each isolate of white-rot macromycetes was obtain from fruiting bodies growing saprophytic, parasitic or both (facultative parasite) on the dead/living hardwood tree. The basidiocarps were identified according to Arora1986 [14], Singer 1986 [15], and Bessette*et al*. 1997[16]. The fruiting bodies collected were surface sterilized by 70 % ethanol then were excised ± 1mm^{3}and inoculated on petri dish contain PDA medium. The plate were incubated for 7 days 28^{o}C.

Dual culture each *Trichoderma* spp. and white-rot macromycetes: A mycelial plug (5 mm diameter) of each macromycetes was placed on one edge of petri dishes (90 mm) containing solid PDA medium. The agar plug was taken from the growing edge of colony. After 3 days incubation (28^{o}C), mycelial plug of *Trichoderma*spp. was put ±4 cm faced to the agar plug containing growing mycelium of macromycetes. The cultures were incubated7x24 days at 28^{o}C.Three replicates were used in each experiment. Petri dishes with agar plug containingindividual mycelia each fungal species were used as control.

Mixed culturetwo species of*Trichoderma* spp. was made by harvesting conidia from each culture of *Trichoderma* spp. on PDA plate. Conidial harvesting was conducted by adding 1 mL sterilized distilled water into 7 days old of the culture. The amount of conidia in the suspension was determined using haemocitometer. Every 0.5 mL conidial suspension of eachspecies of *Trichoderma* was mixed thoroughly with another 0.5 mL conidial*Trichoderma*suspension, and 1 mL of the suspension was cultivated by pour plate method in PDA medium. The culture then were incubated 7 days at 28^{o}C. Agar plug (diameter 5 mm) contain mixed mycelia of two*Trichoderma* spp. was made and used for dual culture with agar plug myceliasingle species of white-rot macromycetes. Three replicates were used for each experiment. In-vitro percentage of inhibition between mycelia of *Trichoderma* spp. and white-rot macromycetes was calculated through formula according to Fokkema 1973 [17].

$$I = \frac{r_1 - r_2}{r_1} \times 100\ \%$$

Where: I = percentage of inhibition

r_1 = radius of the macromycetes away from the *Trichoderma*

r_2 = radius of the macromycetes towards the *Trichoderma*

Colony diameter antagonism both macromycetes and *Trichoderma* spp. were measured as in Figure 1.

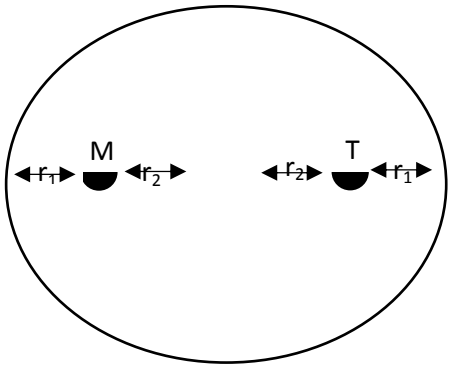


Figure 1. Colony diameter ($r_1 + r_2$) of*Trichoderma* (T) andmacromycetes (M)

RESULTS AND DISCUSSION

After several isolation and purification of soil samples, five isolates of *Trichoderma*spp. were obtained.Each of the isolate was characterizedmacroscopically and microscopically based on colony appearance, growth rate andgrowth pattern,conidiation,conidiophore branching and aggregation, conidia and phialides. All five the unidentified strains of the*Trichoderma* are listed in Table 1.

Table 1.Isolates code of*Trichoderma* spp. isolated from Sibolangit Conservation Forest

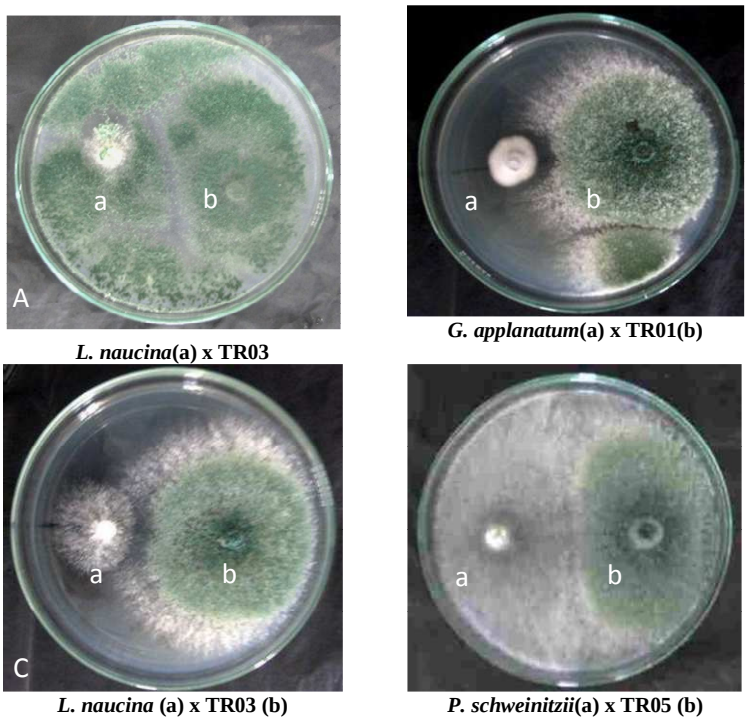
Isolates code	Genus	Origins
TR01	*Trichoderma* spp.1	Sibolangit Conservation Forest
TR03	*Trichoderma* spp.3	Sibolangit Conservation Forest
TR04	*Trichoderma* spp.4	Sibolangit Conservation Forest
TR05	*Trichoderma* spp.5	Sibolangit Conservation Forest
TR06	*Trichoderma* spp.6	Sibolangit Conservation Forest

Based on in-vitro dual culture pre-test indicatescolony diameter all isolates of *Trichoderma* spp.have fast growth, abundant conidiationand higher colony diameter than that of all white-rot macromycetestested. Each species of *Trichoderma* shows antagonistic activity to specific species of white-rot. The different of colonysize and the presence of gap between colonies indicate both *Trichoderma* and white-rot macromyceteshave antagonism characteristic as shown in Table 2 and Figure 2. Colony diameter TR03 and TR01 have≥ 50 mm larger thanfour species white-rot *L. naucina, G. applanatum, T. versicolor* and *C. cinnamomea* respectively. Isolate TR06 reduces colony of *C. cinnamomea, G. applanatum* and *L. naucina*.Among white-rot,*P.schweinitzii*has less inhibited by all tested*Trichoderma* spp.

Table 2. Colony diameter (mm) *Trichoderma* spp. in PDA medium after 7x24 h against white-rot macromycetes

Isolate code	Colony diameter (mm)	Species of Macromycetes	Colony diameter (mm)
TR01	51	*L. naucina*	31
	51	*G. applanatum*	30
	52	*T. versicolor*	36
	51	*C. cinnamomea*	38
	42	*P. schweinitzii*	40
TR03	59	*L. naucina*	28
	57	*G. applanatum*	31
	50	*T. versicolor*	38
	50	*C. cinnamomea*	36
	47	*P. schweinitzii*	41
TR04	46	*L. naucina*	26
	45	*G. applanatum*	31
	47	*T. versicolor*	41
	53	*C. cinnamomea*	35
	44	*P. schweinitzii*	45
TR05	51	*L. naucina*	34
	48	*G. applanatum*	30
	50	*T. versicolor*	37
	47	*C. cinnamomea*	28
	42	*P. schweinitzii*	36
TR06	50	*L. naucina*	30
	50	*G. applanatum*	35
	48	*T. versicolor*	43
	53	*C. cinnamomea*	34
	46	*P. schweinitzii*	43

The mean percentage inhibitionsome of single culture *Trichoderma* spp. to the growth of five species white-rot macromycetes on potato dextrose agar medium (PDA) 7 days after incubation showed thatmycelia growth of *P. schweinitzii*are has less inhibited by strains of *Trichoderma*. The presence of *Trichoderma* have no effect on colony growthof the macromycetes(shown in Figure 2D).



***L. naucina*(a) x TR03** ***G. applanatum*(a) x TR01(b)**

***L. naucina* (a) x TR03 (b)** ***P. schweinitzii*(a) x TR05 (b)**

Figure 2. Single culturemacromycetes (a) and *Trichoderma* spp. (b) in PDA, 7 days incubation (28^{o}C)

Everyspecies of *Trichoderma* spp. has different ability to inhibit the mycelia growth of macromycetes as shown in Figure 3. Almost isolates of *Trichoderma* inhibit the growth *L. naucina* and *G. applanatum*. More than 80 % growth inhibition of *L. naucina*occur by TR03.*Ganoderma. applanatum*is inhibited ≥ 60 % except by TR04. Colonization of *Trichoderma*rapidly grown over the white-rot is antagonism actionto reduce the growth of white-rot mycelia. All isolates of *Trichoderma* spp. have equal ability (40-60 %) to inhibit *C. cinnamomea*and *T. versicolor*. Among white-rot,*P.schweinitzii*has the lowest inhibition, the highest inhibition of all *Trichoderma* isolatesto the macromycetestake place up to 40 % by TR01.

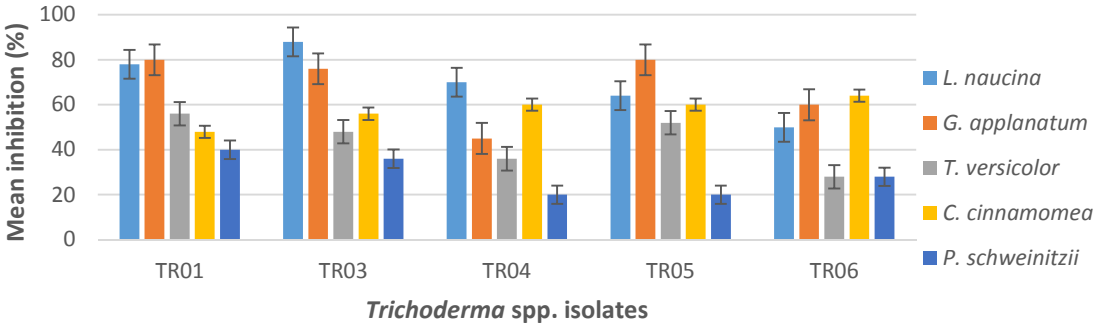


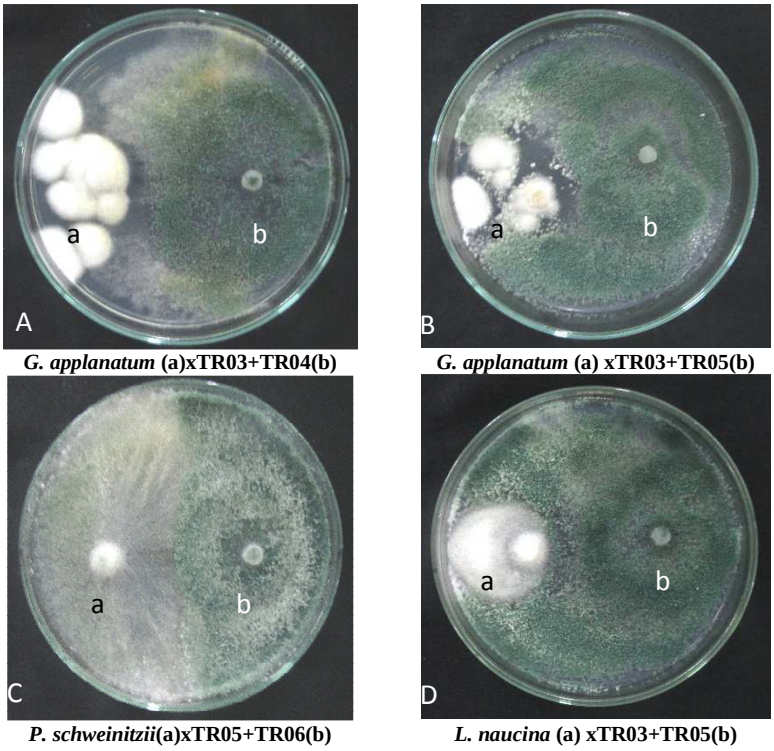
Figure 3. The inhibition percentage of *Trichoderma* spp. (TR01, TR03, TR04, TR05, TR06) against 5 species of macromycetes

The mean percentage inhibition of dual mixed*Trichoderma* spp. against mycelia growth of five species white-rot macromycetes is shown in Table 3.

Table 3. Colony diameter mixed culture of *Trichoderma*spp. against white-rot macromycetes

Isolates code *Trichoderma* spp.	Colony diameter (mm)	Species of Macromycetes	Colony diameter (mm)
TR01 + TR03	60	*L. naucina*	30
	55	*G. applanatum*	32
	45	*T. versicolor*	42
	50	*C. cinnamomea*	40
	42	*P. schweinitzii*	32
TR01 + TR04	49	*L. naucina*	35
	44	*G. applanatum*	29
	43	*T. versicolor*	30
	45	*C. cinnamomea*	44
	37	*P. schweinitzii*	46
TR01 + TR05	45	*L. naucina*	32
	51	*G. applanatum*	29
	45	*T. versicolor*	43
	45	*C. cinnamomea*	43
	43	*P. schweinitzii*	45
TR01 + TR06	54	*L. naucina*	33
	53	*G. applanatum*	18
	45	*T. versicolor*	40
	48	*C. cinnamomea*	38
	40	*P. schweinitzii*	47
TR03 + TR04	50	*L. naucina*	34
	48	*G. applanatum*	25
	45	*T. versicolor*	41
	45	*C. cinnamomea*	37
	37	*P. schweinitzii*	48
TR03 + TR05	60	*L. naucina*	30
	50	*G. applanatum*	30
	53	*T. versicolor*	36
	45	*C. cinnamomea*	45
	41	*P. schweinitzii*	45
TR03 + TR06	57	*L. naucina*	37
	50	*G. applanatum*	19
	46	*T. versicolor*	42
	51	*C. cinnamomea*	33
	46	*P. schweinitzii*	44
TR04 + TR05	50	*L. naucina*	31
	45	*G. applanatum*	16
	49	*T. versicolor*	41
	50	*C. cinnamomea*	35
	38	*P. schweinitzii*	45
TR04 + TR06	54	*L. naucina*	32
	46	*G. applanatum*	26
	41	*T. versicolor*	38
	47	*C. cinnamomea*	35
	38	*P. schweinitzii*	45
TR05 + TR06	41	*L. naucina*	30
	50	*G. applanatum*	33
	40	*T. versicolor*	38
	50	*C. cinnamomea*	39
	42	*P. schweinitzii*	38

Most mixed culture of *Trichoderma* reduces colony growth of white-rot (Figure 4A, B, D). However, mycelia synergism of mixed*Trichoderma* spp. to reduce white-rot is less than that single culture.Compare to the other white-rot, *Phaelusschweinitzii*still less inhibitedby mixed*Trichoderma* spp. as shown in Figure 4C.



G. applanatum **(a)xTR03+TR04(b)** ***G. applanatum*** **(a) xTR03+TR05(b)**

P. schweinitzii**(a)xTR05+TR06(b)** ***L. naucina*** **(a) xTR03+TR05(b)**

Figure 4. Mixed culture of *Trichoderma* spp. (b) and macromycetes species in PDA medium, 7 days incubation (28°C)

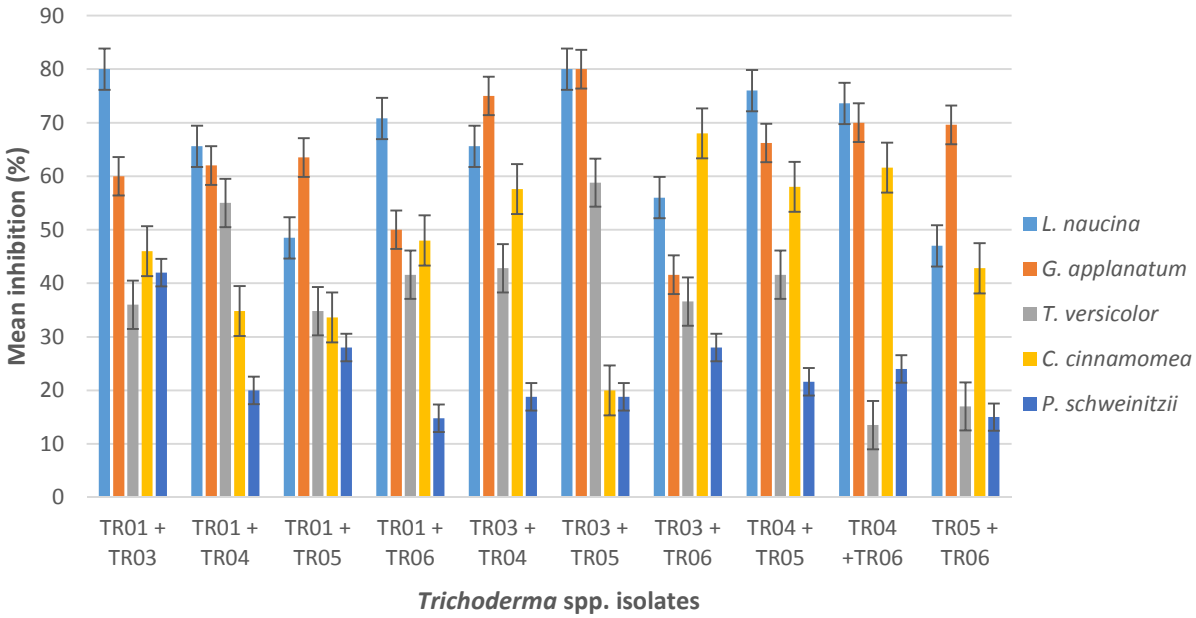


Figure 5. The inhibition of mixture mixed cultureof *Trichoderma* spp. against 5 species white-rot macromycetes on PDA medium after 7x24 h incubation (28°C)

Fast growing myceliaP. *schweinitzii* over grow to*Trichoderma* spp.as indicated by loose mycelial colony rexture, lead to the white-rot macromycetes has less affected on the presence of *Trichoderma*.In contrast to mycelia texture of *G.applanatum* and *L. naucina* (Figure 4A-4D) that have slow growing and compact colony.In Figure 5 showed thateachcombination has antagonism with varying degree inhibition to white-rotmacromycetes. *Lepiotanaucina* and *G. applanatum* are the most inhibited by all combination, followed by *C. cinnamomea. T. versicolor*, and *P. schweinitzii*respectively.Even though competitive ability single culture of *Trichoderma* against the white-rot lower than that of mixed culture, similar pattern of mean inhibition percentage between single culture (Figure 3) and mixed culture (Figure 5) was shown. The ability of *Trichodema* spp. against white-rot macromycetesoccurred in*Trichoderma* TR01, TR03, TR05 and their mixed culture.

Trichoderma has potential as bio-control agent for wide range of fungal plant pathogen [17]. The ability of *Trichoderma* to reproduce asexual by conidia makes the fungus has high competitive. Tronsmo 1986 [7],KaewchayandSoytong 2010 [8] observed rapid colonization of *Trichoderma* spp. as one of antagonism characters to the other fungi. In our study found that shorter and dense diameter colony of white-rot macromycetes(except *P. schweinitzii*) than that of *Trichoderma* indicates white-rot lost growthcompete to the all *Trichoderma* isolates. Lytic enzymes produce by *Trichoderma* destroy cell wall of other fungi was studied bySchirmböck*et al*. 1994 [19] and Kumar *et al*. 2012 [11]. The presence of gap area between colonies indicates some *Trichoderma*TR01, TR03 (Fig. 2B, 2C) and mixed culture TR03+TR04, TR03+TR05 (Fig. 4A, 4B) have enzymatic antagonism. Schubert *et al.* 2008 [10] report wood decay fungi have different susceptibility when challenged by *Trichoderma*. Mixture of two species of *Trichoderma*against basidiomycete indicates most white-rot macromycetes had not completely inhibited [20, 21]. Similar result we found in Table 3. We assumed intra species competition between *Trichoderma*, mixed culture lessen their competitive ability compare to single species against white-rot.Research conducted by Highley and Ricard 1988 [5]stated *Trichoderma* has little protection against white-rot fungi, and*Trichoderma* spp. have variation in antagonistic properties [6].

CONCLUSION

Five isolates of*Trichoderma* spp. were screened from Sibolangit Conservation Forest, North Sumatera. Each of the isolate exhibit characteristic to inhibit the growth of white-rot macromycetes.In-vitro screening and antagonistic test of the fungus showed single species and mixed culture of *Trichoderma*havedifferent percentage inhibition to the growth of the tested white-rot macromycetes.

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