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An Assay on Potential of Local *Trichoderma* Spp. to Control White Root Rot Disease Caused by *Rigidoporus microporus* in Rubber Plant Stump

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White root rot disease caused by *Rigidoporus microporus* was a detrimental disease of rubber plant. In controlling the disease, synthetic chemicals were applied. This control may cause environmental pollution and health problem. In this study, an alternative to control WWRD using biological control agent of local *Trichoderma* spp. isolates was examined. *Trichoderma* spp. were isolated from soil of healthy rubber, sugarcane and tobacco plantation, and of Sibolangit Forest Park. *R. microporus* was isolated from infected rubber tree. Sixteen *Trichoderma* spp. were isolated and subjected to antagonisms assay againt *R. microporus*. *Trichoderma* spp. isolates showed to have different ability in inhibiting *R. microporus* growth. Four isolates i.e. KA03 of rubber, TB03 of sugarcane, TM01 of tobacco plantations, and HU01 of Sibolangit Forest Parks showed relatively higher percentage of inhibition to *R. microporus* by 67.2, 66.3, 70.2, and 71%, respectively. Examination of inhibition of white root rot disease in rubber stump in polyethylene plastic bag showed that the four isolates were able to reduce disease intensity and severity of *R. microporus* after 60 days of control, but no improvement of stump performance was observed.

Keywords: Biological control, *Rigidoporus microporus*, *Trichoderma*.

Indonesia is the world's second largest rubber producer after Thailand. Indonesian rubber exports showed significant progress annually and contribute in raising revenue for the country (Ministry of Agriculture, 2014). However, rubber production faces a significan problem due to rubber plant diseases which causes economic losses not only because of the production loss, but also the high cost required in control the disease.

White root rot disease (WRRD), caused by *Ridigoporus microporus* is one of detrimental disease of rubber tree in Indonesia, India, Malaysia, Sri Lanka, Thailand, West and Central Africa (Kaewchai & Soytong, 2010). WRRD may lead to death of rubber plants. Plants of two to six years are particularly susceptible to this disease. After initial infection this disease may kill three-year old plants within six months and six-year old plant within 12 months, respectively, depends on number of the pathogen in soil.WRRD often causes damage to where there are many root stump or residual wood, and of sandy or loose soil (Situmorang & Budiman, 2003).

Early symptom of WRRD showed as decaying roots of rubber plant attacked, following by pale yellowing leave, folded leaf edges and ends, and sometime with early flowers and fruit

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appearance. White thread of rhizomorph is seen in the root, sometime with yellowish/orange fruiting bodie, mainly at the collar of the dead infected tree. Secondary symptoms such as increasing the number of branches bearing fruit earlier are frequently observed. Leaves of affected plants turn yellow and fall which is followed by the death of the plant twigs (Anwar, 2006).

To control WRRD, an integration of cultural and chemical methods such as removal and burning of the infected root, applying the chemical fungicides has been applied, but sometimes it is too late to control disease (Kaewchai *et al*., 2009). The cultural control may be loborous, while the chemical control may cause environmental pollution and health problems. Biological control is an alternative in controlling the disease, which is expected to reduce the reliance on synthetic chemical compounds. Biological control agent using facultative parasitic fungi such as *Trichoderma* spp. may be used to control the disease. *Trichoderma* spp. the very common and antagonistic fungi found in the soil are well-known as biological control agent of plant fungal disease (Benítez *et al*., 2004; Ikediugwu & Monday 2012; Jeyaseelan *et al*., 2012). This fungus with the help of their enzymes and toxic compounds damages its host and absorb food from the host cells by entering hyphae to the host (Benítez *et al*., 2004; Vinale *et al*., 2008).

Biological control of many plant fungal diseases using *Trichoderma* spp. has been widely reported. Some studies indicate that *Trichoderma* suppressed the growth of pathogens in both the leaf and root. For examples, Jeyaseelan *et al*. (2012) reported the ability of *Trichoderma* spp. to suppress the growth of *Pythiuma phanidermatum* soil borne diseases on tomato plants. Suppression *Collelotrichum capsici* agen of anthracnose disease of chilli using *Trichoderma* has also been reported (Ajith & Lakshmidevi, 2010). So far, almost no study on the isolation and utilization of local *Trichoderma* spp. in North Sumatra especially in controlling WRRD of rubber plants caused by *R. microporus* was reported. In this study, isolation of *Trichoderma* spp. of soil of healthy rubber, sugarcane and tobacco plantation, and from Sibolangit Forest Park was conducted. The isolates were then examined *in vitro* against *R. microporus*, followed by an *in vivo* examination on rubber stump infected with *R. microporus* in polyethylene plastic bag.

MATERIALS AND METHOD

Isolation and characterization of *Rigidoporus microporus*

Suspected fungal infected root as well as fungal mycelia of the infected root was taken. The root was sliced to about 0.5 cm long. Infected roots and mycelia were put on PDA. Cultures were incubated at ambient temperature for 48 hours. Growing colonies was separated to get single colony. Morphological and microscope observation and characterization were conducted to the fungal colony.

Isolation and characterization of *Trichoderma* spp.

Soil samples were taken from healthy rubber, sugarcane, and tobacco plantations, and from Sibolangit Forest Park. The soil samples were cleaned from large particles such as roots and leave debris. Isolation of *Trichoderma* spp. were conducted using dilution method on a common fungal medium, Potato Dextrose Agar (PDA). Soil was diluted with sterile distilled water. One ml of soil dilution was spreaded on PDA added with 50 µg of chloramphenicol. Selected fungal colony was grown in PDA. All cultures were incubated at ambient temperature for 48 hours. Morphological and microscope observation, and characterization of the fungal colony were conducted.

Assay of antagonism of *Trichoderma* spp. againt *Rigidoporus microporus in vitro*

Dual culture method was utilized to examine the ability of *Trichoderma* spp. to inhibit *R. microporus* growth. Actively growing hyphae of *Trichoderma* spp. and *R. microporus* on agar were taken with a 5 mm cork borer. Both fungal hyphae were grown side by side with a distance of 3 cm on PDA. Culture was made by growing single fungus on PDA. Cultures were incubated at ambient temperature. Hyphal diameter of both colonies was measured for 7 days. Percentage of inhibition was measeured as:

$$PIRG=\frac{R_1-R_2}{R_1} \times 100\%$$

PIRG = Percentage of inhibition of radial growth
R1 = hyphal diameter of *R. microporus*

Table 1. Morphological dan microscopic observation of *Trichoderma* spp. characteristics

Soil Camples of	Isolate Code	Morphological characteristics				
		Structure	Color	Shape	Type of growth	Conidiofore
Rubber plantation	KA01	Smooth-cottonlike	White-greenish	Circular	With growing circle	Branched, pyramide-like
	KA02	Granulated	Greenish	Circular	With growing circle	Branched, pyramide-like
	KA03	Smooth-cottonlike	Green	Circular	With growing circle	Branched, pyramide-like
	KA04	Granulated	White-greenish	Circular	With growing circle	Branched, pyramide-like
	KA05	Granulated	White-greenish	Circular	Without growing circle	Branched, pyramide-like
Tobacco plantation	TM01	Granulated	Green	Circular	With growing circle	Branched, pyramide-like
	TM02	Granulated	White-greenish	Circular	Without growing circle	Branched, pyramide-like
	TM03	Granulated	Greenish	Circular	With growing circle	Branched, pyramide-like
	TM04	Granulated	Greenish	Circular	Without growing circle	Branched, pyramide-like
	TM05	Granulated	White-greenish	Circular	With growing circle	Branched, pyramide-like
Sugarcane plantation	TB01	Granulated	White-greenish	Circular	Without growing circle	Branched, pyramide-like
	TB02	Granulated	Greenish	Circular	Without growing circle	Branched, pyramide-like
	TB03	Smooth-cottonlike	Green-yellowish	Circular	Without growing circle	Branched, pyramide-like
Sibolangit Forest Park	HU01	Smooth-cottonlike	Greenish	Circular	Without growing circle	Branched, pyramide-like
	HU02	Smooth-cottonlike	White-greenish	Circular	Without growing circle	Branched, pyramide-like
	HU03	Granulated	Green-yellowish	Circular	Without growing circle	Branched, pyramide-like

Table 2. Reduction of WRRD severity and intensity by *Trichoderma* spp. isolates

Treatments	Disease severity after			Disease intensity after			Severity/recovery level		
	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
(-) control	0	0	0	0	0	0	No infection observed	No infection observed	No infection observed
(+) control	2	3	3	50	100	100	Severely rotted	Heavily rotted	Heavily rotted
KA03	1	0	0	15	0	0	Moderate recovery	Moderate recovery	Moderate recovery
TM01	1	0	0	20	0	0	Moderate recovery	Well-recovered	Well-recovered
TB03	1	0	0	17.5	0	0	Moderate recovery	Moderate recovery	Moderate recovery
HU01	1	0	0	17.5	0	0	Moderate recovery	Well-recovered	Well-recovered

growing as a control

R2 = hyphal diameter of *R. microporus* in dual culture with *Trichoderma* spp.

To observed antagonism effect of *Trichoderma* spp. on *R. microporus*, slide culture was utilized for 7 days. Contacted hyphae were observed microscopically.

Assay on control of WWRD on rubber stump

Selected *Trichoderma* spp. isolates were subjected to growth in autoclaved rice media. Rubber stump with disease intensity of 25-50% of 1 month old were grown in polyethylene plastic bag of 30 x 40 cm. *Trichoderma* application of 50 g/stump was conducted after 1 week of the growth of rubber stump by pouring *Trichoderma* rice culture on digged soil around rubber stump. Poured *Trichoderma* culture was re-covered with soil. Observation was conducted on disease intensity by digging soil around stump 5-10 cm depth. Disease intensity was measured as 0% = no disease, 1-25% = mild, 25-50% = moderate, 50-75% = severe, and 75-100% = highly severe intensity, while disease severity was measured on a scales of 0 = no disease, 1 = light, 2 = moderate, and 3 = severe infection.

RESULT

Isolation and characterization of *Rigidoporus microporus*

Rigidoporus microporus isolated from infected rubber root showed to have white flattened cottony-like colony, smoothy mycelium with no ring of growth observed (Figure 1.). In 4 days of incubation, the colony overgrew of 9

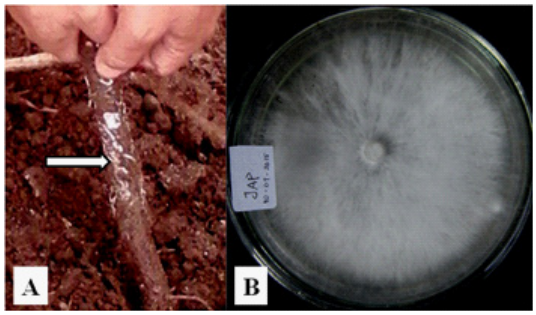

Fig. 1. A. Infected rot (arrowed) with hyphae of *R. microporus*, and B. Overgrown colony of *R. microporus* in petri dish

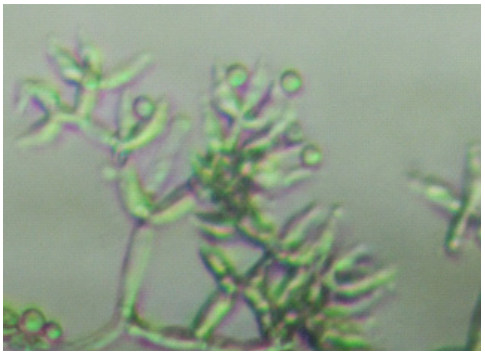

Fig. 2. Conidia of one isolate of *Trichoderma*

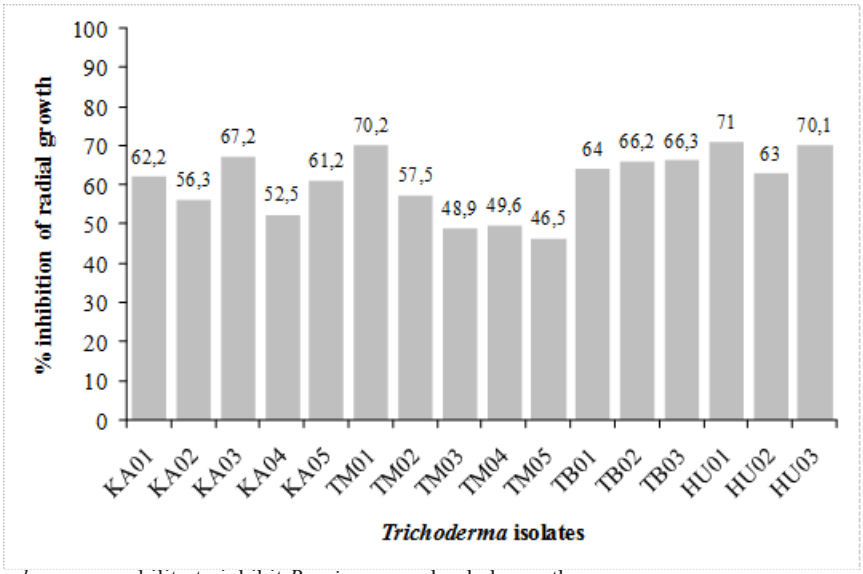

Fig. 3. *Trichoderma* spp. ability to inhibit *R. microporus* hyphal growth

cm petridish on PDA. Hyphae was septate with hyaline basidiospore with diameter of 3.28 ìm. These characters were similar to that of Kaewchai *et al*. (2009) observation on *R. microporus* isolated from South part of Thailand.

Isolation and characterization of *Trichoderma* spp.

Sixteen *Trichoderma* spp. were isolated from different soil samples, i.e. 5 isolates of rubber (KA01, KA02, KA03, KA04, KA05), 3 isolates of sugarcane (TB01, TB02, TB03), 5 isolates of tobacco plantations (TM01, TM02, TM03, TM04, TM05), and 3 isolates of Sibolangit Forest Park (HU01, HU02, HU03). Morphological observation showed that isolates KA01, KA03, TB03, HU01 and HU02 have smooth and cottony colony surface and isolates KA02, KA04, KA05, TM01, TM02, TM03, TM04, TM05, TB01, TB02, and HU03 were granulated. The isolates had a variety in colors such as white-green, light green, green, and green-yellowish, and spherical shape of colony. Seven colonies grew laterally with ring of growth, however, others did not show ring of growths. These features belong to *Trichoderma* spp. Extended growth showed to have dark green colony (Table 1.). Microscopic observation of the hyphae showed that conidiophore was with pyramide-like branch (Figure 2.) as specific to *Trichoderma* (Samson *et al*., 1995).

Table 3. Stump condition and root number of rubber stump after treated with *Trichoderma* spp.

Treatments	Stump Survival	Root Number
(-) control	Alive	5
(+) control	Death	0
KA03	Alive	1
TM01	Alive	0
TB03	Alive	1
HU01	Alive	1

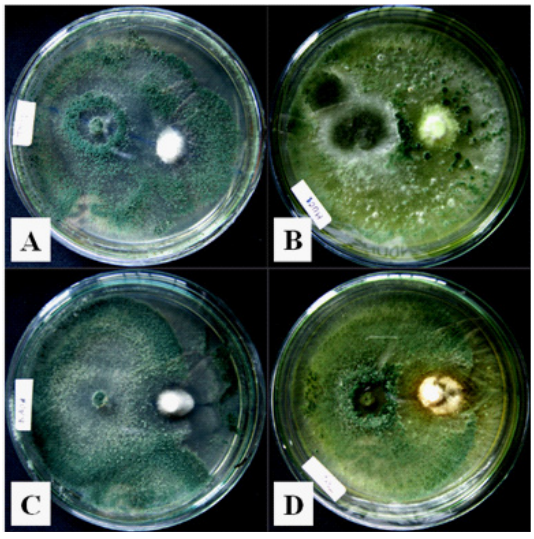

Fig. 4. *Trichoderma* spp. hyphae overgrown on *R. microporus*

Assay of antagonism of *Trichoderma* spp. to *Rigidoporus microporus in vitro*

In vitro antagonism assay was conducted using a dual method on PDA. Observation of growth inhibition on *R. microporus* was carried out in 2-8 days of incubation. *Trichoderma* spp. ability to inhibit *R. micoporus* growth varied, four potential isolates were to showed to inhibit more (Figure 3.). Antagonistic effect was observed on 2-days of incubation, but clearly seen on 4-days of incubation, in which *R. micoporus* was inhibited by *Trichoderma* spp. At the end of observation, *Trichoderma* spp. growth almost over entire dish, suppressing *R. microporus* growth (Figure 4.). Microscope observation of four potential isolates of *Trichoderma* spp. showed that their hyphae penetrated and wrapped *R. microporus* hyphae around (Figure 5.). Kubicek *et al*. (2001) reported that mycoparasitism is one of the most common mechanism showed by *Trichoderma* spp. After recognizing the host, *Trichoderma* hyphae attached to host hyphae by twisting and then penetrate host cell wall through secretion of cell wall degrading enzymes (Viterbo *et al*., 2002). Mycoparasite

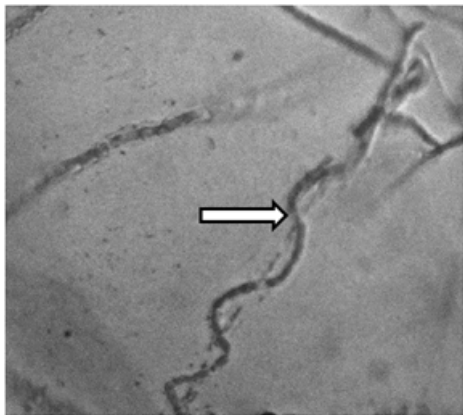

Fig. 5. *Trichoderma* spp. hyphae twisted *R. microporus* hyphae around

producing cell wall degrading enzymes is to perforate host hyphae to takes nutrients from host and uses it for growing. Chitinase and â -1,3-glucanase has been observed to get directly involved in mycoparasitic mechanism between *Trichoderma* spp. with the host (Kubicek *et al*., 2001), or antibiosis, antibiosis by producing an antibiotic 6-pentyl-a-pyrone (6PP), and peptaibol heptilidic acid (Vinale *et al*., 2008), competition for nutrients and space, and induce local and systemic resistance of the plant (Harman, 2006).

Reduction of WRRD severity and intensity by *Trichoderma* spp. isolates

Trichoderma isolates with relatively higher inhibition zone from different locations i.e. KA03, TM01, TB03 and HU01 were used in examination to reduce WWRD intensity in rubber stump *in vivo*. Observations were carried out every 30 days of 90 days. In all observation, a decline of disease severity and intensity, and improving rubber stump healthy of all *Trichoderma* treatments was observed compared to that of (+) control (Table 2.). Number of rubber stump survival and its root were also observed. Number of rubber stump survival was seen by making small wound to rubber stump to see a greeny tissue, while number of root was observed by gently digging soil around growing stump. The result showed that *Trichoderma* spp. isolates was not able to enhance stump performance, such as root number, however, all *Tricoderma* isolates were able to keep the stump alive (Table 3.). It seemed that the effect of *Trichoderma* spp. in increasing plant performance might not occur immediately.

DISCUSSION

WRRD caused by *R. microporus* is an important disease in rubber. The disease attacks all stage of rubber plants, especially to newly rubber plantation, and may cause high economic losses compared to other diseases. Situmorang (2004) reported that dry latex production declines at least 2.7 kilograms/tree/year. This disease attacks the roots by forming an overgrown flattened mycelia strands called rhizomorf of white or white-yellowish threadlike resembling root hairs, and later on develops to form its fruiting bodies. Infected trees show a general foliage discoloration, proceed sometimes by premature flowering and fruiting (Omorusi, 2012). Root eventually rots and the rubber plant crashes. Rotting of the roots is due to destruction of chemical structure of wood as a result of fungal enzyme activity. In addition, mechanically penetration through colonized natural openings or wounds were also contribute to plant damage (Omorusi 2012).

In vitro examination of *Trichoderma* spp. isolates showed to inhibit *R. microporus* hyphae *in vitro. Trichoderma* is known as effective biological control agents for many plant fungal diseases, and also were known for promoting plant growth (Ikediugwu & Monday 2012; Benítez *et al*., 2004). Biological control activity of *Trichoderma* occurs directly and indirecty through several mechanisms competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, and by mechanisms such as mycoparasitism (Howell, 2003; Diby *et al*., 2005; Benítez *et al*., 2004**;** Harman, 2006; Ikediugwu & Monday 2012).

Kubicek *et al*. (2001) reported that mycoparasitism is one of the most common mechanism showed by *Trichoderma* spp. After recognizing the host, *Trichoderma* hyphae attached to host hyphae by twisting and then penetrate host cell wall through secretion of cell wall degrading enzymes (Viterbo *et al*., 2002). Mycoparasite producing cell wall degrading enzymes is to perforate host hyphae to takes nutrients from host and uses it for growing. Chitinase and â -1,3-glucanase has been observed to get directly involved in mycoparasitic mechanism between *Trichoderma* spp. with the host (Kubicek *et al*., 2001).

Penetration of some *Trichoderma* hyphae into infected plant tissue might inhibit the pathogen growth, and might recover to plant growth (Harman *et al*., 2004). In this study, eventhought *Trichoderma* spp. application decreased disease severity and intensity of WRRD as well, but it seemed that the isolates could not completely recover the stump that was previously infected with *R. microporus* with moderate disease intensity of 25-50%. Application of biological control agents prior fungal pathogen infected the plant possibly shows a different result.

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