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EFFECT OF TEMPERATURE AND STORAGE ON EFFECTIVENESS OF *Trichoderma viride* AS BIOCONTROL AGENTS OF *Rigidoporus microporus,* PATHOGEN OF WHITE ROOT ON RUBBER

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ABSTRACT

This research was aimed to study the effect of temperature and storage on effectiveness of *Trichoderma viride* to suppress *Rigidoporus microporus* development, the pathogen of white root disease on rubber. This research applied a randomized complete block design with five treatments and five replications. Each treatment contained two rubber plants (total of plants 50). There were five treatments i.e.: compost containing *T. viride* which was stored at 17^{o}C for a month (A), compost containing *T. viride* which was stored at 24^{o}C for a month (B), compost containing *T. viride* which was stored at 30^{o}C for a month (C), Triadimefon (D) and *R. microporus* (control Results showed that *that T. viride* compost which was stored for a month at various temperatures was able to suppress *R. microporus* development. *Trichoderma viride* compost which was stored at of 17^{o} C for a month showed the best result. It was was able to supress white root disease severity up to 70 percent and rhizomorph colonization up to 62 percent respectively. The treatment was also able to increase the plant height and stem diameters.

Keywords: effectiveness, *Rigidoporus microporus*, storage duration, temperature, *Trichoderma viride*

INTRODUCTION

Indonesia is the second largest rubber producer after Thailand. There are 3.4 million ha spread over several provinces with the total production of 3.04 million tons (Anonymous, 2013). *Rigidoporus microsporus* is known as one of important pathogens which attacks rubber plantation and causes low production (Jean and Albert, 2002; Jayasurija and Thennakoon, 2007; Situmorang *et al*., 2007; Jayasinghe, 2010).

Fungal mycelia of *R. microsporus* penetrate directly into the root tissue. Each rubber plant attacked by this pathogen will die if not immediately addressed. Died plant will be a source of inoculum for healthy plants in the vicinity. Loss of production due to this pathogen attack on rubber planting can reach 5-15 % annually (Guyot and Flori, 2001; Judawi *et al.*, 2006).

Since long time, control over this disease has been emphasize more on the use of chemicals or fungicides, but it has been realized that the use of persistent chemicals can result in a negative effect on the environment. The use of chemical fungicides could adversely affect human health and the importance of microorganisms in the soil, pathogen resistance to chemicals, pollution, and it could leave residue in soil (Haggag and Mohamed, 2007; Kim and Hwang, 2007).

One step that can be developed potentially in the future is utilizing antagonistic microorganisms. *Trichoderma* spp has been well known to control many plant diseases including white root disease in the rubber plant, without causing negative effects on the environment. Some species of Trichoderma such as *T. viride, T. koningii and T. virens* are known to control the white root disease (Hightley, 1997; Jayasuriya and Thennakoon, 2007). In Indonesia, Trichoderma application in rubber is usually in the form of compost. Generally farmers do not directly use the compost but kept it for half to a month in the storage. Related to white root disease on rubber, it's important to evaluate aspects of temperature and storage periods on the effectiveness of Trichoderma. This research was aimed to study the effect of temperature and storage periods on the ability of *T. viride* compost to suppress the development of white root disease on rubber.

MATERIALS AND METHODS

This research was carried out in a green house, Inderalaya, Ogan Ilir Dstrict, South Sumatra, Indonesia from January to August 2012. The research used a completely randomized block design with five treatments and five replications and each treatment contains 2 rubber plants (total of plants were 50). The treatments were: *T. viride* compost stored at 17ºC for a month (A), *T. viride* compost stored at 24ºC for a month (B), *T. viride* compost stored at 30ºC for a month (C), Triadimefon (D) and *R. microporus* (control). Rubber seedlings used were clones of PB 260 aged 8 months from seed, which were grown in soil mixed with sand and cow manure (1:0.5:1). A total of one petri dish *T. viride* inoculum was propagated in the Glucose Yeast media and than put into 500 gr compost mix of sawdust, manure and rice bran (1:0.6:0.4). The mixture was then stirred until smooth then stored according to the treatment. Propagation of *R. microporus* was done by mixing as many as 5 pieces of cork drill *R. microporus* inoculum on root rubber sticks and than stored in sterile bottles.

Application *T. viride* was given by sprinkling about 2 grams of compost which contained *T. viride* surrounding the root rubber plant 15-25 mm in depth and then covered again with soil. Inoculation of pathogen was done by inserting 4 sticks of rubber roots containing *R. microporus* into the soil around the plant rubber 5 days after the application of Trichoderma compost. Observed parameters include the severity of the disease, colonization rhizomorph on the ground, plant height and stem diameter. Data were then analyzed by analysis of variance (ANOVA), which then was followed by Honestly Significant Difference Test (HSDT) comparison among means.

RESULTS AND DISCUSSION

Diseases severity

The results showed that the storage of *T.* viride compost at various temperatures affected the severity of white root disease of rubber plants (Figure 1). The effect of each treatment was very good when compared to the control, but the best treatment was *T. viride* compost that was stored at 17ºC for one month. This treatment wasn't different from the results indicated by chemical treatment (Triadimefon) that were used as chemical control, a commonly used chemical by farmers in southern Sumatra, Indonesia for controlling white root disease in rubber.

Severity of the disease seen in the treatment of *T. viride* compost stored at 17°C for a month was significantly different from *T. viride* compost treatment stored at 24°C for a month and 30°C for a month as well as the control (Table 1). The lowest disease severity on the treatment of *T. viride* compost stored at 17°C for a month was 12.19% and the highest 40.23% on control. This treatment was able to suppress the white root disease severity up to 70% compared to control.

Table 1. Effect of *T. viride* compost stored at various temperatures on white root disease severity on rubber

Treatments	Disease severity (%)
T. viride compost stored at 17ºC for a month (A)	12.19 (70.0) a
Triadimefon (D)	22.92 (43.0) ab
T. viride compost stored at 24ºC for a month (B)	26.38 (34.4) b
T. viride compost stored at 30ºC for a month (C)	28.11 (30.1) bc
Rigidoporus microporus (control)	40.23 (100) c

Remarks: numbers followed by the same letters are not significantly different. Numbers in brackets are relative values compared to control

Figure 1. Effect of *T. viride* compost on *R. microporus* the pathogen of white root disease on rubber. a) *T. viride* compost stored at 17^{o}C for a month, b) *T. viride* compost stored at 24^{o}C for a month, c) *T. viride* compost stored at 30^{o}C for a month, d) Triadimefon and e) *R. microporus* (control).

The ability of *T. viride* in suppressing the white root disease severity was presumably triggered by the antagonist grown at temperature of 17^{o}C, so it brought good suppressing ability. According to Samuel *et al.* (2005), *T. viride* can grow well at the optimum temperature of 15^{o}C–30^{o}C. Trichoderma can infect its host through direct growth, contacts, host recognition, attachment of trichoderma hyphae on the host hyphae, forming appressorium on the host surface, penetration and exit. Attachment of hyphae was facilitated by binding carbohydrates in the cell walls of Trichoderma fungi with lectins on the host (Whipps, 2001; Harman *et al*., 2004). Once it is in contact, Trichoderma produces antibiotic and toxins and cell wall enzymes destroyer (Kubicek *et al*., 2001; Harman *et al*., 2004). Trichoderma species not only has a variety of metabolites which are toxins, but also has a variety of enzymes such as exo- and endoglucanase, cellobiase, chitinase, cellulase and protease. These enzymes can destroy host wall structures (Fox, 2003).

Rhizomorph Colonization on Media

Trichoderma viride compost was significantly different from each other on *R. microporus* colonization on media (Table 2). Table 2 shows the effect of treatment on *R. microporus* colonization rhyzomorph on the media by Triadimefon (D), *T. viride* compost which was stored at 17°C for a month, which was not significantly different from *T. viride* compost stored at 24°C for a month. *T. viride* compost stored at °C 24 was, however, significantly different from treatment of *T. viride* compost stored at 30°C for a month and control. *T. viride* compost which was stored at 17°C for a month was able to suppress the rizomorph colonization on media up to 62 % compared to control.

Trichoderma is antagonistic agent that has a capability to produce metabolic compounds, destroy, and inhibit other microorganisms. In addition, Trichoderma is able to compete with plant pathogen in nutrition uptake which causes the inhibition of plant pathogen such as *R. microporus* (Elad and Freeman, 2002). Trichoderma produces volatile substances such as gas (including acetaldehyde, n-propanol, propional, isobutanol, n-butyraldehyde, ethyl acetate, isobutyl acetate, acetone) which can inhibit the growth of *R. solani, P. domesticum*, *M. hiemalis*, *P. ultimum*. Several proteolytic enzymes produced by Trichoderma play an important role in the destruction of the fungus *Sclerotium rolfsii. Trichoderma viride* produces two antibiotics such as gliotoxin (toxic against *R. solani*) and gliovirin (toxic to *Phytium spp*) (Howell, 2003). This ability is believed to be able to inhibit rhizomorph colonization of *R. microporus* in soil.

Rubber Plant Height Stem Diameter

Composting *T. viride* on rubber plants affected plant height and stem diameter. The treatment of *T. viride* compost which was stored at 24^o and 30^{o}C in a month (B and C) was not significantly different from control, but

Triadimefon and *T. viride* compost stored in a month at 17ºC (A) were significantly different from control (Table 3 and Table 4).

Table 2. Effect of *T. viride* compost stored at various temperatures on *R. microporus* rizomorf colonisation on media

Treatment	Rizomorf colonization on media (%)
Triadimefon (D)	12.93 (66.8) a
T. viride compost stored at 17ºC for a month (A)	14.79 (62.0) ab
T. viride compost stored at 24ºC for a month (B)	25.97 (33.4) ab
T. viride compost stored at 30ºC for a month (C)	31.55 (19.1) bc
Rigidoporus microporus (control)	39.00 (100) c

Remarks: numbers followed by the same letter are not significant different. Numbers in brackets are relative values compared to control

Table 3. Effect of *T. viride* compost on rubber plant height

Treatment	Rubber plant height (cm)
Rigidoporus microporus (control)	10.00 a
T. viride compost stored at 24ºC for a month (B)	11.10 a
T. viride compost stored at 30ºC for a month (C)	16.70 ab
Triadimefon (D)	22.00 b
T. viride compost stored at 17ºC for a month (A)	22.50 b

Remarks: numbers followed by the same letters are not significantly different

Table 4. Effect of *T. viride* treatment on increasing rubber stem diameters

Treatment	Stem diameter (mm)
Rigidoporus microporus (control)	0.64 a
T. viride compost stored at 24ºC for a month (B)	0.82 ab
T. viride compost stored at 30ºC for a month (C)	0.87 ab
Triadimefon (D)	0.95 bc
T. viride compost stored at 17ºC for a month (A)	1.07 bc

Remarks: numbers followed by the same letters are not significantly different

A number of *Trichoderma* species are successfully used as a biological control agent because they grow fast, have high productivity, have diverse control mechanisms, are excellent competitors in the rhizosphere, tolerant, or resistant to fungicides, strong aggressive against phytopathogenic fungi, and promote plant growth (Benítez *et al*., 2004). In addition, Trichoderma is known to promote the development of root which can increase the plants biomass and productivity, increase the ability to absorb nutrition, and have efficiency of nitrogen usage. Those abilities can also dissolve the nutrients in the plant tissues (Harman *et al.*, 2004). According to Howell (2006), Trichoderma has the capacity to make rizospher colonization and promote plant growth.

CONCLUSIONS

Trichoderma viride compost stored for a month at various temperatures was able to suppress the development of *R. microporus* and accelerate the rubbers plant height and diameter significantly compared to control and traidimefon. The best treatment was *T. viride* compost which was stored at 17º C for a month that was able to suppress the white root disease severity up to 70 percent and capable of suppressing the rizomorph colonization on media up to 62 % respectively compared to control.

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