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

Nurhayati Damiri¹, Mulawarman and Mitra Mutiara

Department of Plant Pests and Diseases, Faculty of Agriculture, Sriwijaya University
Jl. Raya Palembang-Prabumulih, Km 32, Ogan Ilir, Inderalaya 30662, South Sumatra, Indonesia

¹Corresponding author Phone: +62-711-580663 Email: nurhayatidamiri@yahoo.co.id

Received: March 31, 2014 / Accepted: July 16, 2014

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°C for a month (A), compost containing T. viride which was stored at 24°C for a month (B), compost containing T. viride which was stored at 30°C for a month (C), Triadimefon (D) and R. microporus (control). Results showed 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 17°C for a month showed the best result. It was able to suppress 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 microporus is known as one of important pathogens which attacks rubber plantation and causes low production (Jean and Albert, 2002; Jayasri and Thennakoon, 2007; Situmorang et al., 2007; Jayasinghe, 2010). Fungal mycelia of R. microporus 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; Jayasri 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 greenhouse, Inderalaya, Ogan Ilir District, 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 17°C for a month (A), *T. viride* compost stored at 24°C for a month (B), *T. viride* compost stored at 30°C 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 g 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 g 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 17°C 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.

| Treatments                                      | Disease severity (%) |
|------------------------------------------------|----------------------|
| *T. viride* compost stored at 17°C for a month (A) | 12.19 (70.0) a       |
| Triadimefon (D)                                  | 22.92 (43.0) ab      |
| *T. viride* compost stored at 24°C for a month (B) | 26.38 (34.4) b       |
| *T. viride* compost stored at 30°C 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°C for a month, b) *T. viride* compost stored at 24°C for a month, c) *T. viride* compost stored at 30°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°C, so it brought good suppressing ability. *T. viride* can grow well at the optimum temperature of 15°C–30°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 (Kubíček *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 rhizomorph 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-butyaldehyde, 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 rizomorph 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°C and 30°C in a month (B and C) was not significantly different from control, but Triadimefon and *T. viride* compost stored in a
month at 17°C (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* rizomorph colonization on media

| Treatment | Rizomorph colonization on media (%) |
|-----------|-------------------------------------|
| Triadimefon (D) | 12.93 (66.8) a |
| *T. viride* compost stored at 17°C for a month (A) | 14.79 (62.0) ab |
| *T. viride* compost stored at 24°C for a month (B) | 25.97 (33.4) ab |
| *T. viride* compost stored at 30°C 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 significantly 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 24°C for a month (B) | 11.10 a |
| *T. viride* compost stored at 30°C for a month (C) | 16.70 ab |
| Triadimefon (D) for a month (A) | 22.00 b |
| *T. viride* compost stored at 17°C for a month (A) | 22.60 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 24°C for a month (B) | 0.82 ab |
| *T. viride* compost stored at 30°C for a month (C) | 0.87 ab |
| Triadimefon (D) for a month (A) | 0.95 bc |
| *T. viride* compost stored at 17°C 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 rizosphere 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 rubber's plant height and diameter significantly compared to control and triadimefon. The best treatment was *T. viride* compost which was stored at 17°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.

**REFERENCES**

Anonymous. 2013. Agricultural investment opportunities in Indonesia. Directorate of Investment and Business Development, Directorate General of Processing and marketing for Agricultural Product, Ministry of Agriculture. Republic of Indonesia. pp. 10.

Benitez, T., M.A. Rincon., M.C. Limon and C.A. Codon. 2004. Biocontrol mechanisms of Trichoderma strains, International Microbiology 7(4): 249-260.

Elad and Freeman. 2002. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy and fluorescence microscopy. Phytopathology 73 (1): 85-88.
Fox, R. T. V. 2003. Managing Armillaria root. Food, Agricultural and Environment. 1(1): 95-100.

Guyot, J. and A. Flori. 2001. Comparative study detecting *Rigidoporus lignosus* on rubber trees. Crop Protectio 21(6): 461-466.

Haggag, W.M. and H.A.A. Mohamed. 2007. Biotecnological aspects of microorganisms used in plant biological control. American-Eurasian Journal of Sustainable Agriculture 1(1): 7-12.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chef and M. Lorito. 2004. *Trichoderma* species - opportunistic, avirulent plant symbionts. Nature Review Microbiology 2: 43-56.

Hightley, L.T. 1997. Control of wood decay by *Trichoderma* (Gliocladium) virens I. Antagonistic properties. Material and Organism 31(2): 79-89.

Howell, C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant disease: The history and evolution of current concepts. J. of Plant Disease. 87(1): 4-10.

Howell, C.R 2006. Understanding the mechanism employed by *Trichoderma* spp to the effect biological control of cotton disease. Phytopathology 96(2): 178-180.

Jayasinghe, C.K. 2010. White root disease of rubber tree: An overview. Paper presented in The Proceeding of The International Workshop on White Root Disease on Hevea Rubber. p.1-8.

Jayasuriya, K.E. and B.I. Thennakoon. 2007. Biological control of *Rigidoporus microporus*, the cause of white root disease in rubber. Ceyon Journal of Science (Biology and Science) 36(1): 9-16.

Jean, G. and Albert, F. 2002. Comparative study for detecting *Rigidoporus lignosus* on rubber trees. Crop Protection. 21(6) 416-466.

Judawi, S.D., L. Holomoan and R.B. Setyaningsih. 2006. A guide for plant pest and disease control on rubber plant. Directorate General of Estate, Ministry of Agriculture, Republic of Indonesia. Jakarta. p.1-3.

Kim, B.S and B.K. Hwang. 2007. Microbial fungicides in the control of plant diseases. Journal of Phytopathology 155: 641-653.

Kubicek, C.P., R.L. Mach, C.K. Peterbauer and M. Lorito. 2001. *Trichoderma*: From genes to biocontrol. J. of Plant Pathology. 83 (2): 11-23.

Situmorang, A., H. Suryaningtyas and S. Pawirosoemardjo. 2007. Current status of white root disease (*Rigidoporus microporus*) and the disease control management in rubber plantation. Pp. 27-33. in Pawirosoemardjo, S., B. Setyawan., H. Suryaningtyas., M. Supriadi. Proceeding of International Workshop on White root Disease of Hevea Rubber. Salatiga 28th – 29th November 2006.

Whippys, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. J. of Experimental Botany 52(2): 487-511.