

# THE PREVENTIVE CONTROL OF WHITE ROOT ROT DISEASE IN SMALL HOLDER RUBBER PLANTATION USING BOTANICAL, BIOLOGICAL AND CHEMICAL AGENTS

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## ABSTRACT

**The preventive control of white root rot disease in small holder plantation using botanical, biological, and chemical agents.** A field and laboratory experiment were conducted from June 2008 to December 2009 in Panumangan, Tulang Bawang - Lampung. The field experiment was intended to evaluate the effect of botanical plants (*Alpinia galanga*, *Sansiviera auranthii*, and *Marantha arundinacea*), biological agents (organic matter and *Trichoderma* spp.), and chemical agents (lime and natural sulphur) on the incidence of white root rot disease and population of some soil microbes. The laboratory experiment was conducted to observe the mechanism of botanical agents in controlling white root rot disease. In the field experiment, the treatments were applied in the experimental plot with cassava plant infection as the indicator. The variables examined were the incidence of white root rot and population of soil microbes. In the laboratory experiment, culture of *R. microporus* was grown in PDA containing root exudate of the antagonistic plant (botanical agent). The variable examined was colony diameter of *R. microporus* growing in the PDA plates. The results of the field experiment showed that planting of the botanical agents, and application of *Trichoderma* spp., as well as natural sulphur, decreased the incidence of white root rot disease. The effectiveness of *M. arundinacea* and *Trichoderma* spp. was comparable to natural sulphur. The laboratory experiment showed only root exudate of *A. galanga* and *S. auranthii* that were significantly inhibit the growth of *R. microporus*.

Key words: white root rot, rubber, cassava, *Alpinia galanga*, *Sansiviera auranthii*, *Marantha arundinacea*, *Trichoderma*, organic matter, lime

## ABSTRAK

**Pengendalian preventif penyakit akar putih di perkebunan rakyat menggunakan agensia botanis, biologis, dan kimiawi.** Percobaan lapangan dan laboratorium telah dilakukan dari bulan juni 2008 sampai dengan bulan Desember 2009 di Panumangan, Lampung. Percobaan lapangan ditujukan untuk mengetahui pengaruh agensia botanis (*Alpinia galanga*, *Sansiviera auranthii*, and *Marantha arundinacea*), biologis (bahan organik dan *Trichoderma* spp.), dan kimiawi (kapur dan belerang) terhadap penyakit akar putih dan populasi beberapa mikroba tanah. Percobaan laboratorium ditujukan untuk mengamati kemampuan tanaman antagonis dalam menekan penyakit akar putih. Pada percobaan lapangan bahan-bahan diaplikasikan pada plot percobaan dengan tanaman singkong sebagai indikator infeksi. Variabel yang diamati adalah insiden penyakit akar putih dan populasi mikroba. Pada percobaan laboratorium, kultur *R. microporus* ditumbuhkan pada media PDA yang mengandung eksudat tanaman antagonis; variabel yang diamati adalah diameter koloni *R. microporus*. Hasil percobaan lapangan menunjukkan bahwa tanaman antagonis dan *Trichoderma* secara nyata menekan insiden penyakit akar putih. Dari hasil percobaan di laboratorium diketahui bahwa hanya eksudat akar tanaman *A. galanga* dan *S. auranthii* yang secara nyata menghambat pertumbuhan *R. microporus*.

Kata kunci: Akar putih, karet, singkong, *Alpinia galanga*, *Sansiviera auranthii*, *Marantha arundinacea*, *Trichoderma*, bahan organik, dan kapur.

## INTRODUCTION

White root rot disease (*Rigidoporus microporus*) is very serious disease in rubber plantation. The disease was reported as a serious threat since it causes significantly yield loss. The disease causes loss on production about 3-15% with the financial value about

2.1 billion rupiahs every year particularly in small holder plantation. The damage in small holder plantation occurred often in the areas where stumps of rubber and primary forest trees exist, and in sandy and loose soil areas (Widjanarko, 1969; Basuki, 1981; Basuki, 1982; Situmorang & Budiman, 1990).

In small holder rubber plantations, control of white root rot disease is rarely conducted so that it caused high incidence of the disease in those areas. Some of the reasons were due to the lack of farmers' knowledge both on the disease and the control methods, and the lack of farmer's income. Therefore it is necessary to introduce alternative control methods suitable to the social and economic condition of the rubber farmers.

In the control of white root rot disease, preventive efforts is much better than curative efforts such as using fungicides. The preventive controls of white root rot disease consist of the elimination of infection sources and crop protection. The most effective infection source elimination recommended until now is by throwing of the stumps mechanically and the elimination of the roots residu from the plantation areas (Fox, 1977; Liyanage *et al.*, 1984). However, this is difficult to be applied by rubber small holders because of the high cost. Other method of stumps eliminatain suitable to the farmers is the use of some chemicals to poison old stumps (Fox, 1977; Basuki, 1981), burning of old stumps, growing of antagonistic plants (Martin & du Plessix 1969; Situmorang & Suryaningtyas, 2003), and the use of decomposer fungi (Situmorang, 1996). The most effective protection is the use of synthetic fungicides (Situmorang & Budiman, 1990; Situmorang, 1994a) but this method is expensive because of the high price of the synthetic fungicides. Other control methods cheaper than the use of synthetic fungicides and more suitable to small holder farmers are the use of natural sulphur (Azaldin, 1985; Basuki, 1986), application of *Trichoderma* spp. (Soepena *et al.*, 1992; Situmorang, 1994b) and the use of botanical agents (antagonistic plant) (Situmorang & Suryaningtyas, 2003). This study was conducted to evaluate the effects of botanical agents (*Alpinia galanga*, *Sansevieria auranthii*, and *Marantha arundinacea*), biological agents (organic matter and *Trichoderma* spp.), and chemical agents (natural sulphur and lime) on white root rot incidence and some soil microbes population; and to envestigate the role of botanical agents on white root rot incidence.

## MATERIALS AND METHODS

Laboratory experiment was conducted in the Laboratory of Plant Disease, Faculty of Agriculture, University of Lampung from July to October, 2009. Plant root exudates were collected from each of the rhizosphere of the botanical agents tested. The PDA medium containing 300 ppm of streptomycine was used to culture *R. microporus*. As much as 1 ml of root exudate was spread on the surface of each petri plate.

The variable observed was diameter colony of *R. microporus* at three and six days after investation.

A field experiment was conducted in small holder rubber plantation in Penumangan Baru Village, Central Tulang Bawang District, Tulang Bawang regency, Lampung Province, from June 2008 to December 2009. This field experiment was intended to observe the effect of botanical agents, organic matters, *Trichoderma* spp., natural sulphur, and lime on the incidence of white root rot disease and some soil microbes population. This field experiment consisted of eight treatments arranged in randomized complete block design with three replicates. The treatments were three botanical agents (galangale, sansivera, and *Marantha arundinacea*) grown in the rubber field; *Trichoderma* spp., organic matter, and two chemical agents (natural sulphur and lime) applied in the rubber field, and a control. The *Trichoderma* spp. (approx.  $10^3$  spores per gram rice brand) were obtained from Sembawa Rubber Research Center. The incidence of white root rot infection was observed on cassava cuttings that were planted in the experimental plots as the indicator plant. Three tested botanical agents were grown around the cassava cuttings. The biological agents (*Trichoderma* spp. and organic matters) were applied on the soil surface around cassava cuttings as much as 100 gram per cutting. Variables examined were white root rot incidence on the cassava cuttings and abundance of soil microbes including mycorrhiza, nematodes, and nonpathogenic soil fungi (fungi other than white root rot fungi). For the observation of soil microbes, composited soil samples as much as 1,0 kg were collected from each sampling point of each plot, within the depth of 0 – 20 cm.

**White Root Rot Incidence Observation.** Within every experiment plot, the number of normal or healthy growing cassava cuttings and wilting cassava cuttings were counted. White root rot disease incidence were determined using the formula as follow:

$$DI = \frac{n}{N} \times 100\%$$

Where DI = white root rot disease incidence,

n = number of wilting cassava, and

N = number of total population of cassava plants per plot observed.

**Mycorrhizal Fungi Observation.** Extraction of mycorrhizal spores was conducted using sieving and decanting method. As much as 50 g soil was put in a plastic jar (2 L), added by 300 cc tap water, and mixed thoroughly for several seconds to detach mycorrhizal

spores (such as spores of *Glomus manihotis*) from the roots. Let the suspension in 1 minute to deposit the coarse particles before decanting using sieving tools with 250, 100, and 38  $\mu\text{m}$  of pore diameter. During sieving, repeated washing were conducted using flowing water. The retained aliquot of the smallest sieving tool was placed in beaker glass using 100 cc of flowing water. The aliquot was mixed with glass rod and placed in centrifuge tubes (50 cc). As much as 25-30 cc of sugar dilution (60% b/v) was placed in the base of the centrifuge tubes using plastic syringe. Centrifuge tubes were centrifuged with the speed of 2900 – 3000 rpm in 2 minutes. Mycorrhizal spores were collected using 38  $\mu\text{m}$  sieving tool and washed with flowing water to free the spores from sugar. Using spraying bottle mycorrhizal spores were stored in grid line Petri dish for the population counting under stereoscopic microscope (40x). Spores with the same morphology were taken with micro pipette and grouped for the genus identification. Mycorrhizal spores were identified until the level of genus using the identification book on VAM fungi (Schenk & Perez, 1990).

**Nematode Observation.** Nematode extraction on 300 cc of soil samples were conducted using varied level of sieving tools and then centrifused in sugar dilution (500 g in 1 l solvent) according to Gafur & Swibawa (2004). Laboratory process and the enumeration of abundance and diversity of soil nematodes is the same as explain in community survey of nematode in method of plant parasitic nematode management on the coffee base agricultural land.

**Observation of Fungi other than *R. microporus*.** The observation of nonpathogenic fungi abundance was conducted using plate-counting methods. Soil sample was serial diluted and from each of dilution level 1 ml was spread on media containing rose-bengal chloramphenicol. The observation was conducted by grouping, counting, and identifying fungal colony with the same morphology. Identification of fungi was conducted at genus level based on fungal morphology.

## RESULTS AND DISCUSSIONS

The results of the field experiment showed that *Sansevieria* sp., *A. galanga*, *Trichoderma* spp., natural sulphur, and *M. arundinacea* significantly decreased the disease incidence of white root rot on cassava plants (Table 1). The results also showed that soil microbes population were affected by the treatments, and later they may affect the white root rot incidence.

*Trichoderma* spp. decreased soil fungi population (Figure1), but *M. arundinacea* increased soil fungi population. It is known that *M. arundinacea* tubers contain carbohydrates that act as the source energy for most soil fungi. In this experiment, the effectiveness of *M. arundinacea* in suppressing the incidence of white root rot is tend to be better than natural sulphur. Beside that, all treatments decreased the population of nematodes but did not affected the population of mycorrhizal fungi.

*Trichoderma* spp. was significantly decreased (82%) the incidence of white root rot on the indicator plants. It is known that *Trichoderma* spp. are antagonist fungi against many pathogenic fungi (Monte, 2001). The work of Jayasuriya and Thenakoon (2007) showed that *Trichoderma* isolates obtained from rubber growing areas were antagonistic to *R. microporus*. The mechanism of *Trichoderma* spp. in controlling plant disease is very complex. *Trichoderma* spp. was widely reported as the best antagonist fungi since its ability in competition for nutrients and space, producing some enzymes and toxic substance or antibiotics, and also act as mycoparasite (Tahia *et al.*, 2004).

Organic matter tended to decrease the white root rot incidence on cassava plants as the indicator plant. This result indicated that organic matter has many factors potentially suppressed the growth of *R. microporus*. Organic matter has a role as the food base of some soil microbes (Lewis & Papavizas, 1984) that are being antagonist of *R. microporus*. Another possible reason why organic matter tended to decrease white root rot incidence is related to parasitic nematodes population. It is known that under the organic matter influence, the total number of nematode population significantly decreased compared to control (Figure1). As in many cases, nematodes have a role as agravator for plant disease, including white root rot. The decrease of total nematode population may due to the occurrence of the pathogen of nematode, i.e. *Arthrobotrys* spp. Effects of organic matter was statistically not different. Organic matter was reported can supply high nitrogens, and this condition will increase the activity of laccase (D'Souza *et al.*, 1999) resulted in the increase of wilt incidence.

Lime treatment was also tended to decrease (32%) white root rot incidence. It indicated that lime affected the growth of *R. microporus*. The result of previous study showed that the increase of pH in acid soil promoted the population of antagonistic fungi against *R. microporus*, and finally suppressed the incidence of white root rot (Prasetyo *et al.*, 2009). The white-root rot fungus prefers porous soil with netral pH (around 6-

Table 1. The disease incidence of white root rot observed on cassava as the indicator plant

| Treatments                  | White root rot incidence (%) |
|-----------------------------|------------------------------|
| Control                     | 18.7 a                       |
| Lime                        | 12.7 ab                      |
| Organic Matter              | 10.0 abc                     |
| <i>Sansevieria</i> sp.      | 8.0 bc                       |
| <i>Alpinia galanga</i>      | 4.7 bc                       |
| <i>Trichoderma</i> spp.     | 3.3 bc                       |
| Natural Sulphur             | 2.7 bc                       |
| <i>Marantha arundinacea</i> | 1.0 c                        |

Numbers in the column followed by the same letter are not significantly different according to LSD test at  $\alpha = 0.05$ .

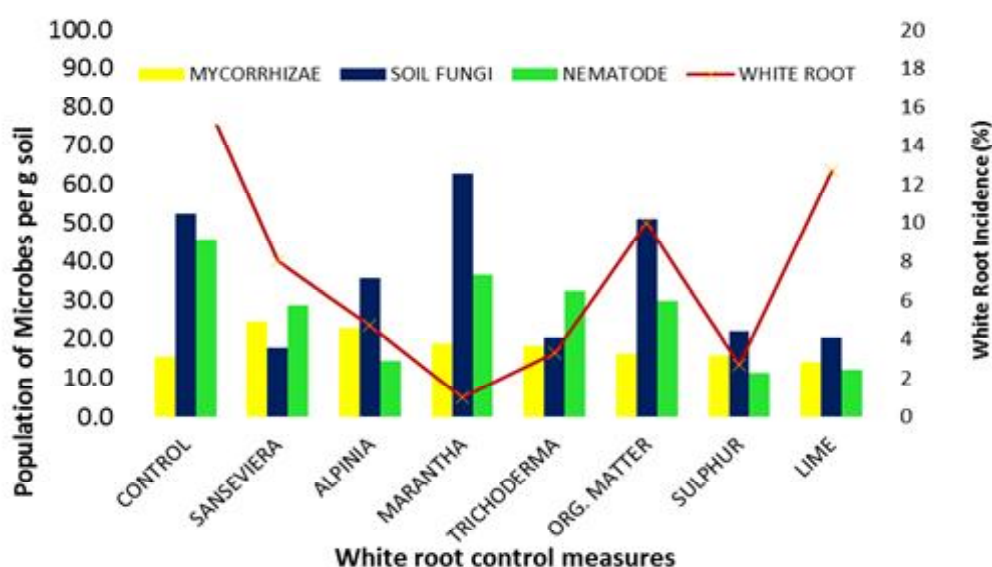


Figure 1. Effect of plant root exudates on white root rot incidence and population of soil microbes

7), including in liparite soil in East Sumatra and Southern Mountains in East Java (Semangun, 2000). In South Sumatra this disease was commonly found in small holder rubber plantation grown in sandy soil (Situmorang and Budiman, 1990). This demonstration plots (acidic soil, pH 4 – 4.04) showed that the increase in pH correlated with the decrease in the white-root disease incidence (Prasetyo *et al.*, 2009). This correlation seemed to be indirect, possibly through the increase of soil fungi population and diversity.

Botanical agents such as *M. arundinacea*, *A. galanga*, and *Sansevieria* sp. significantly decreased the incidence of white root rot on cassava cuttings as the indicator plants in the field. The results of the laboratory study, also showed that root exudates of *Marantha arundinacea*, *A. galanga* and *S. auranthii* decreased the colony diameter of *R. microporus* (Table 2). It was known that *A. galanga* and *Sansevieria* sp. produce toxic substance and produce essential oil that toxic to most of fungal taxon. Marziana (2008) reported

that root exudates of those two plants effectively suppressed the in vitro growth of *R. microporus*.

In the field study, *M. arundinacea* was the most effective in decreasing the incidence of white root rot disease (Table 1, Figure 1). In the laboratory study, *M. arundinacea* did not significantly suppress the growth of *R. microporus* at three days after incubation but later on six days after incubation *M. arundinacea* caused the best suppression of *R. microporus* growth (Table 2). It was assumed that *M. arundinacea* planted in the soil that resulted in the suppression of white root rot incidence was in correlation to the fact that the population of soil fungi was the highest in the rhizosphere of *M. arundinacea* (Figure 1). Root exudates of *M. arundinacea* that apparently contain carbohydrates may enhance the growth of soil fungi and increase their population. It is very likely that the increase of soil fungi population was in line with the increase of the population of antagonistic fungi (Figure 2) and resulted in the suppression of *R. microporus* growth. It is worthy to

Table 2. Effect of plant root exudates on colony diameter of *R. microporus*

| Root exudates of      | Colony diameter of <i>R. microporus</i> |        |
|-----------------------|---|--------|
|                       | 3 days                                  | 6 days |
| <i>M. arundinacea</i> | 2.83 ab                                 | 3.58 c |
| <i>S. auranthii</i>   | 2.68 b                                  | 4.98 b |
| <i>A. galanga</i>     | 2.64 b                                  | 4.72 b |
| Control               | 3.10 a                                  | 6.47 a |

Numbers in the column followed by the same letter are not significantly different according to LSD test at  $\alpha = 0.05$ .

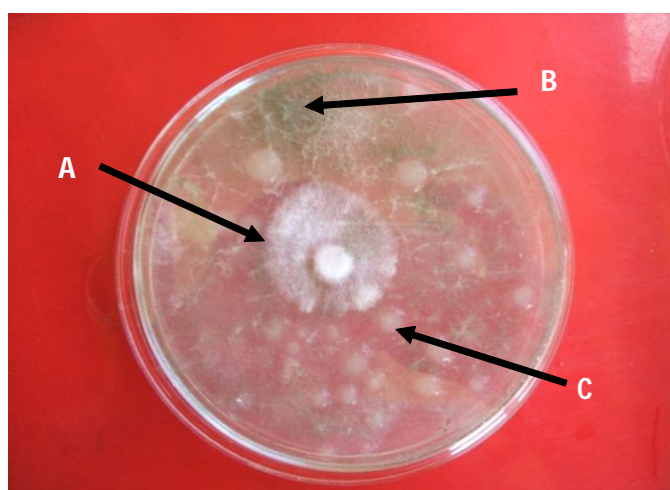


Figure 2. Culture growing on PDA media containing *Maranta* root exudates. (A) *R. microporus*, (B) *Trichoderma* spp., and (C) large colony of yeast.

## CONCLUSIONS

Application of natural sulphure, *Trichoderma* spp. and planting *A. galanga*, *S. auranthii* and *M. arundinacea* decreased the incidence of white root rot disease in the rubber field. In the laboratory, *A. galanga* and *S. auranthii* and *M. arundinacea* decreased the colony diameter of *R. Microporus*. In the culture containing exudates of *M. arundinacea* grew *Trichoderma* spp. and yeast, and the growth of *R. microporus* was suppressed.

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