

# Detection of White Root Disease (*Rigidoporus Microporus*) in Various Soil Types in the Rubber Plantations Based on The Serological Reaction

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## Abstract

The Conventional detection of White Root Disease (*Rigidoporus microporus*, WRD) still uses the visual method based on an abnormal color of leaf or mycelium growth on the tap root neck. The method was less effective and less efficient. The serological technique uses yolk chicken antibodies induced by immunization with mycelium extract. The purpose of this research was to examine the consistency of selected antibodies in detecting root fungi at various soil types in the rubber plantations. This research used a Completely Randomized Design non-factorial with twelve (12) treatments and two (2) replications. The results showed that the antibodies could detect WRD in various soils types. The serological detection was higher precisely than visual observation. The development of WRD mycelium varies depending on the soil types and it was different in the each estate area. In addition, this research is expected to get a serology kit to detect early symptoms of WRD in the rubber plants.

Keywords: *Hevea brasiliensis*, White Root Disease, Early Detection, Serological Technique,

## 1. Introduction

White root disease caused by the fungus *Rigidoporus microporus* is one of the most important diseases in rubber plants in Indonesia. The financial loss due to this disease is very high, especially in smallholder rubber plantations (Setyawan, et al., 2013). Therefore, the preemptive effort through early detection will be more effective and economically viable than curative approach. Early detection of WRD disease symptoms is still difficult to be done. Observation of leaf symptoms is the first method to identify plants primarily for young plants. The plant canopy color change indicator indicates that the pathogen attack has already reached to the heavy stage.

The Conventional method for WRD detection has been developed since 1930. Napper (1932) and Pichel (1956) recommended detection by soil removal around the collar to expose a length of the tap and lateral roots. Declert (1960) proposed the use of log traps to detection of WRD mycellium development. Martin (1964) reported good and economical results with detection by mulching, which induces rhizomorphs to grow from the superficial contaminated rhizosphere. The result of Guyot and Fluri (2002) research explain than a good detection of diseased trees is needed especially in young crops (< 2 years after planting). Detection by infrared photography to characterize spectral changes between the reflectance of healthy and infected rubber tree crowns is unsuccessful (Nandris et al., 1985).

The serology test by using antibodies in the serum and it can detect pathogen microorganism (Fang & Ramasamy, 2015). The specific discovery of antibodies to target diseases is essential to facilitate



diagnosis. Dalimunthe et al., (2016) has obtained antibodies that can recognize the presence of WRD in the soil and WRD infections through WRD infected leaf extract. Further testing of these antibodies is warranted for the technology. The purpose of this research was to examine the consistency of selected antibodies in detecting root fungi at various soil types in the rubber plantations.

## 2. Material and Methods

The research was conducted in March - June 2017 at the Laboratory Plant Protection, Sungei Putih Research Center. The soil samples were taken from four rubber estate area of PTPN III: Labuhan Haji, South Merbau, Rantau Prapat and Tanah Raja fields respectively.

The research design was a Completely Randomized Design non-factorial with twelve treatments and two replications. The soil was taken 1 m from the neck of the roots at a depth of 0-20 cm. The soils were sterilized at 121<sup>0</sup>C for 30 minutes and incubated at the room temperature for 2 days before it was transferred into each jar bottle weighing  $\pm$  250 gr. Pure culture of *R. microporus* diameter 1 cm<sup>2</sup> was inoculated into each soil and incubated for  $\pm$  3 months. The observed growth of the JAP mycelium was performed visually and qualitatively by using light (+), moderate (++) and heavy (+++) categories. One gram of soil-inoculated WRD mycelium was extracted used General Extract Buffer (GEB) with the addition of 80 mg of polyvinyl pyrrolidone (PVP). The extracts were centrifuged at 11,000 rpm for 15 min at 4OC. The supernatant was used as an antigen source and was given Ag1-Ag12 code. The antibodies used were antibodies immunized with WRD extract (antibody-induced WRD mycelium extract/M.Ab) and have successfully detected WRD mycelium in soil and leaf (Dalimunthe et al., 2016) The antigen is diluted 100 times with PBST for antigen and carbonate coating buffer for antibody dilution. After that, the each antigen and M.Ab were reacted into each cuvet of 750  $\mu$ l. The antigen-antibody reaction was read by a spectrophotometer at a wavelength of 405 nm. The data were analyzed statistically using ANOVA.

## 3. Results and Discussion

WRD development in the soil visually was varied both between plantations and within the plantation itself. The highest WRD developments were in the field of Labuhan Haji and the lowest in Tanah Raja field (Table 1). The development of WRD depends on pH, organic matter content, moisture and aerate soils. WRD grow well on high humidity above 90%, high content of organic matter and good aeration. If this condition is appropriate, WRD may spread as far as 30 cm within 2 weeks (Sinulingga & Eddy, 1989). The result of artificial inoculation WRD shows that mycelium was grown varies on different soil types (Figure 1).

**Table 1.** The development level of white root disease attack qualitatively with artificial inoculation

Location of Sample Soils	Development level of WRD
Soil in Labuhan Haji Field	+++
Soil in Labuhan Haji Field	+++
Soil in Labuhan Haji Field	+++
Soil in South Merbau Field	+++
Soil in South Merbau Field	+++
Soil in South Merbau Field	++
Soil in Rantau Prapat Field	+++
Soil in Rantau Prapat Field	+
Soil in Rantau Prapat Field	+++
Soil in Tanah Raja Field	+
Soil in Tanah Raja Field	++
Soil in Tanah Raja Field	++

Note: + (light), ++ (moderate) dan +++ (heavy)



**Figure 1.** Artificial inoculation of white root disease in twelve soil types as a source of antigen (Ag). WRD's were cultured in the room condition for three months.

The reaction of selected antibodies with Ag1 - Ag12 was measured at a wavelength of 405 nm can be seen in Table 1. All of the antigens reactions were higher five times than negative controls. Increasing of absorbances indicated that of antigen-antibody bonds. The reaction proved that the antibodies recognize WRD mycelium in the soil. Nevertheless, all antigens-antibody reaction was lower than antibody-mycelium extract reactions (Ab-MAg) as positive controls. Based on these reactions can be predicted the development of mycelium in the soil between gardens were varies. Even the development of WRD in the same field there was also varied such as Labuhan Haji, South Merbau, Rantau Prapat and Tanah Raja field. Development of WRD mycelium the highest is known in the soil of Labuhan Haji field with an average absorbance of 0.1430 and the lowest in Tanah Raja field with an absorbance rate of 0.1243. The variation was thought to be related to the chemical and physical properties of the soil.

This level of serology reaction has corresponded to the number of mycelium that develops in the soil. The higher the rate of development of mycelium in the soil the higher the absorbance at 405 nm. Thus, MAb can be accurately recognized mycelium in the soil.

The use of mycelium extracts to produce polyclonal and monoclonal antibodies has been reported to detect the types of mycorrhiza (Gobel et al. 1995) and *Candida famata* (Pisa et al., 2007). The results of Dalimunthe et al. (2016) showed that antibodies of mycelium (Ab) were able to recognize mycelium antigens as positive controls with the absorbance of 0.1307. The next results of studies using selected antibodies were also able to detect mycelium antigens with the absorbance of 0.223 as a positive control and significantly different from negative control with the absorbance of 0.026 (Table 2).

**Table 2.** The reaction of antibodies (Ab) with antigen (Ag) of various soil types inoculated WRD based on absorbance value 405 nm.

Location of Sample Soils	Antigen	Absorbance 405 nm.
Negative Controls	AbBufer	0,026d
Positive Controls	AbAgM (mycelium extract)	0,223a
Labuhan Haji Field	AbAg1	0,1520b
Labuhan Haji Field	AbAg2	0,1350bc
Labuhan Haji Field	AbAg3	0,1420bc
South Merbau Field	AbAg4	0,1440bc
South Merbau Field	AbAg5	0,1340bc
South Merbau Field	AbAg6	0,1310bc
Rantau Prapat Field	AbAg7	0,1420bc
Rantau Prapat Field	AbAg8	0,1230 c
Rantau Prapat Field	AbAg9	0,1360bc

Tanah Raja Field	AbAg10	0,1195c
Tanah Raja Field	AbAg11	0,1240c
Tanah Raja Field	AbAg12	0,1295bc

Figures followed by different letter in the same column are significantly different based on Duncan multiple range test at 5% significant level

#### 4. Conclusions

Serology techniques by utilizing polyclonal antibodies could detect the development of WRD mycelium in various soil types appropriately. Base on the result, the serological test could be developed as an early warning system for WRD infection. The short term output of this research is a serological kit that can use for detecting of WRD early symptoms on infected rubber plant.

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