DETECTION OF WHITE ROOT FUNGUS (*Rigidoporus microporus* (Fr.) Overeem) IN SOIL USING CASSAVA ROOT BAITS

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ABSTRACT

White root fungus (*Rigidoporus microporus* (Fr.) Overeem) devastates thousands of hectare of rubber tree plantation in Indonesia. The information on the presence of inoculum in the soil is important for farmers to make preemptive management action. The objective of the research was to study the use of cassava root as bait to detect *R. microporus* in soil. Assays were conducted in Plant Clinic, IPB University, Bogor, Indonesia from 2012 to 2013. Cassava root was tested as baits, with artificially inoculated soils, at various concentration of inoculum levels (10^2, 10^3, 10^4 cfu/g soil). Frequency of baits colonized by *R. microporus* and other fungi was examined. Moreover, sensitivity test was also made by testing cassava root cut with soil artificially infested by four soilborne pathogenic fungi. Finally, comparison was made between cassava root baits and plating on Worrall selective medium for detection of white root rot fungus from infested field soil. Cassava root baits was proven to be an effective, selective and fairly sensitive technique for the detection of white root fungus. Cassava root baits technique was able to detect the fungus with minimum propagule density of 10^3 cfu/g soil. The technique was comparable with plating on selective Worrall media.

Key words: baiting, inoculum, selectivity, sensitivity, technique.

INTRODUCTION

White root fungus (*Rigidoporus microporus* (*Fr.*) Overeem syn. *Rigidoporus lignosus* (Klotzsch) Imazeki) causes death of rubber trees (*Hevea brasiliensis (*Willd. ex A. Juss.) Muell. Arg.). The disease was reported in most rubber latex producing countries. Symptoms of white root fungus (WRF) can be identified by defoliation of the leaves in one or several branches or the entire canopy, very severe symptoms can cause death of the tree, and the presence of rhizomorph on tree collar and roots (Soytong and Kaewchai 2014). Countries most affected by the disease are Malaysia, Thailand, Sri Lanka, Filipina, and Nigeria, with 5-10 % of cultivated land are infested (Jayasinghe 2011). Loss of production each year due to damage by WRF disease reaches 5-15% (Situmorang and Budiman 2003). Therefore, effective control measures are needed to overcome the disease in rubber latex production. Management control measures require detection techniques that are effective, fast and inexpensive.

Techniques were developed to detect *R. microporus*. including the use of selective medium (Worrall 1991), immunological technique using enzyme-linked immunosorbent assay (ELISA) (Louanchi et al. 1996), and polymerase chain reaction (PCR) (Oghenekaro 2016). However, these techniques cannot be applied directly by smallholders and field agricultural officers in developing countries due to limitation of resources and relatively expensive cost.

R. microporus can be detected directly in the field by digging bait in soil surrounding the tree, then the exposed collar is covered with organic mulch (Guyot and Flori 2002), or placing the baits such as rubber seeds baits or tree shoots into soil near basal stem (Ogbebor et al. 2013). However, if these methods are used throughout a rubber tree plantation, it will be labor intensive. In addition, these direct field detections are not quantitative and have problems related to soil moisture variability when applied in large scale. The detection of *R. microporus* in soil is important to plan and implement mitigation strategies in the nursery and field. A detection technique that can be used in limited or in the absence of standard laboratory equipment is an advantage for such conditions.

Field observation by the authors show that cassava root is frequently colonized by *R. microporus* rhizomorph. Since cassava is abundant where rubber tree and seedlings are cultivated, we recognize the possibility of the use of cassava as bait for detection of *R. microporus*. The study sought to investigate the use of cassava root to detect *R. microporus* in soil as an applicable diagnostic technology in less equipped conditions.

MATERIALS AND METHODS

This research was carried out in the laboratory of Plant Clinic, Department of Plant Protection Faculty of Agriculture, IPB University, Bogor, Indonesia from 2012 to 2013.

Inoculum preparation. An isolate of *Rigidoporus microporus* Rm NS1 collection of Plant Clinic IPB University was used in the experiments. Rm NS1 has been identified based on morphological and molecular characteristics (data not shown). This fungus was cultured in potato dextrose broth (PDB), pH 6.5. Fungal cultures of 7-day old was used in the experiments.

Optimization of soil water content for white root fungus detection. Soil water content was adjusted to 20%, 30%, 40%, and 50% by adding a specific volume of water after gravimetric measurement. The colonization rate of *R. microporus* was assessed in soil with each of the adjusted water content.

Artificial infested soil was made by blending the *R. microporus* grown in PDB using Ultra-Turrax homogenizer at 2000 rpm, for 3 minutes (Zweck et al. 1978). The density of the mycelial suspension was determined by plating in potato dextrose agar (PDA) for 24 hours. The inocula which was kept in the refrigerator was then used to adjust propagule density to 10^5 cfu/g of sterilized soil in a plastic pan (40 cm x 20 cm x 8 cm). Cassava roots cv. Adira were cut into 10 cm length with diameter of approximately 5 cm. Cassava roots were washed under running water, and surface sterilized with sodium hypochlorite (1%) and ethanol (1%), then rinsed with sterilize water. Each treatment was replicated five times with 20 cassava roots per replicate. Baits were incubated for five days. Colonization frequency was determined by percentage of root cut with rhizomorph. Positive colonization of *R. microporus* was indicated by rhizomorph forming on cassava root cut surface and was confirmed by microscopic examination based on morphology as described by Kaewchai et al. (2010), then colonization frequency was determined by calculating percentage of positive colonization.

The experiment was arranged in randomized complete block design, in which water content was treatment with five replications. Data were analyzed statistically using analysis of variance according to SPSS 16.0. The differences of means were identified by DMRT (Duncan Multiple Range Test) Test at 0.05 level.

Sensitivity assay of cassava root bait to *R. microsporus*. Sterilized and unsterilized soil were separately mixed with mycelial suspension of *R. microsporus* and propagule density was adjusted to 10^2, 10^3, 10^4, and 10^5 cfu/g soil. The soil water content was adjusted at 40% w/w at the depth of 10 cm because this is optimum soil moisture for growth of *R. microsporus* in the soil (Parasayu et al. 2016). The mixture of soil with the fungus was placed in a plastic pan (40 cm x 25 cm x 8 cm; WxLxD).

Cassava root baits were surface sterilized and then set at a 5 cm depth in the pan. Pans containing soil and baits were covered with transparent plastic bags.

Baits were incubated for 5 days without watering and determined its recover y rate by the percentage of bait colonized by *R. microsporus*. Colonization determine based on morphology with microscopy examination. Colonization on the baits by other fungi was also recorded to get additional selectivity ability other than test using various soil fungi. Twenty root cuts were used for each block and was replicated five times. The experiment was arranged in factorial randomized complete design. The first factor was soil sterilization using sterilized and unsterilized soil, and the second factor was inoculum density of *R. microporus* $10^{2,}$ 10^3, 10^4, 10^5 cfu/g soil. Data were analyzed statistically using analysis of variance according to SPSS 16.0. The differences of means were identified by DMRT (Duncan Multiple Range Test) Test at 0.05 level.

Selectivity assay of cassava root to *R microporus.* The selectivity of cassava root bait was further tested on some soil-borne fungi such as *Ganoderma boninense, Phytophthora capsici, Fusarium oxysporum,* and *Rhizoctonia solani* from the collection of the Plant Clinic, IPB University. These fungi were cultured in PDB and vortexed. The propagule density of each fungi was adjusted at two levels, 10^3 and 10^4 cfu/g soil. Soil moisture was adjusted at 40% before placing a 10 cm long cassava root with a diameter of about 5 cm. Colonization frequency of all tested fungi was determined by observation at 5 days after treatment. The growth of inoculated fungi on the surface of the bait was regarded as colonization. The experiment was arranged in factorial randomized complete block design. The first factor was tested soil fungi, which consisted of five fungi including *R. microporus*, second factor was inoculum density, 10^3 and 10^4 cfu/g soil. Data were analyzed statistically using analysis of variance according to SPSS 16.0.

Comparison of cassava root baiting and the use of selective media in detecting *R. microporus*. Ten soil samples (500 g each) collected from the rubber tree plantation in IPB University Campus Darmaga Bogor were used to detect *R. microporus,* employing either cassava root baiting or selective media technique using Worrall medium (Worrall 1991). The presence of the fungus was assessed five days after plating. The presence of the fungus on Worrall medium was indicated by the colony characterized by Kaewchai et al. (2010), i.e. white and flattened colony, branched, and no clamp connection. Observed colony was also compared with standard fungal isolates. The recovery rate of the fungus on cassava root bait was determined by estimating the propagule density in Worrall medium. Linear regression on colonization frequency of baiting technique and $\log_{10}$ propagule density of selective medium technique was performed using SPSS 16.0, to assess the comparison of the techniques.

RESULTS AND DISCUSSION

Sensitivity of the technique. Cassava root cut bait was considered fairly sensitive as it was able to detect propagule density of *R. microporus* at quite low level *i.e.* 10^3 cfu/g soil (Table 1). Therefore, the technique has high significance for practical use. The density of the WRF propagules in natural infested was mostly above 10^3 cfu/g soil (Fig 1). In addition, most of the Hymenomycetes fungi had propagule density ranging 10^3-10^6 cfu/g soil (Worrall 1991).

Table 1. Colonization of white root fungus, *Rigidoporus microporus,* on cassava root bait with different propagule density in sterilized and unsterilized soils.

Treatment	**Colonization frequency (%) at different propagule density (cfu/g) soil**			
	10^2	**10^3**	**10^4**	**10^5**
Sterilized soil	0 a	26.25 ± 5.59 b	55.0 ±9.60 c	100±0.00 d
Unsterilized soil	0 a	18.25 ±4.25 b	45.0±6.54 c	100±0.00 d

Numbers followed by same letters are not significantly different by DMRT at P < 0.05

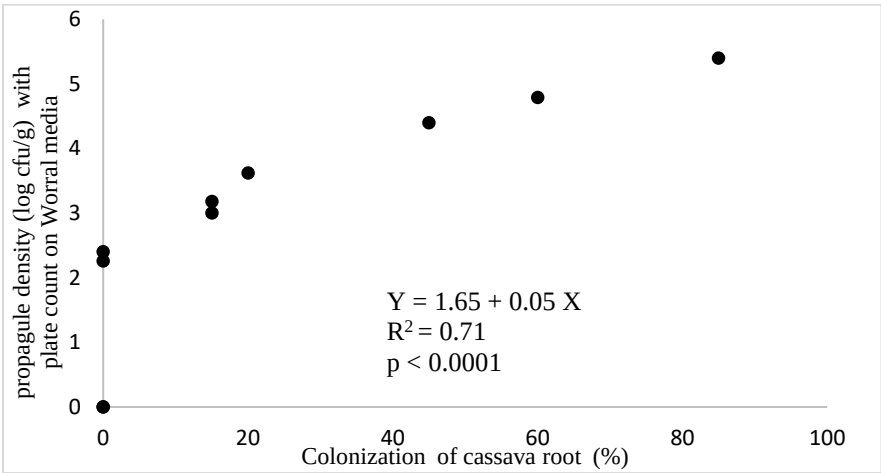

Fig. 1. Relationship between cassava root baiting and selective-medium plating in detecting white root fungus, *Rigidoporus microporus* in soil.

For the detection of WRF, some researchers apply serological (Dalimunthe et al. 2017; Louanchi et al. 1996), and molecular methods (Oghenekaro 2016; Novianti et al. 2017). Even though the technique is sensitive and quick, the usefulness for field worker and farmers in developing countries is limited, due to limitations in appropriate laboratory equipment. The bait technique has been already applid for various soilborne plant pathogens such us *Phytophthora* spp. (Pettitt et al. 2002; Edena et al. 2000), *Pythium* (Sanchez et al. 2000), and *Fusarium oxysporum* f.sp. *melonis (*Palmero et al. 2008). Furthermore, a baiting technique was developed using plant parts for Basidiomycetes fungi such as oak shoot for *Armillaria* spp. (Onozato et al. 2009), and also rubber tree shoots (Guyot and Flori 2002) and seeds for *Rigidoporus microporus* (Ogbebor et al. 2013). Rubber tree shoots (Guyot and Flori 2002) and seeds for *Rigidoporus microporus* (Ogbebor et al. 2013) were also used however, the two latest methods applied in the field required inspection of all trees. Therefore, this is difficult to apply in a large area as it is highly labor intensive and high variation in soil moisture presents additional problems. This technique using cassava roots and adjusted soil moisture overcome the variability of soil moisture and require less labor than the baiting technique *in situ* as described by Guyot and Flori (2002).

Selectivity of cassava root bait. An important advantage of a detection technique is its selectivity. Cassava root baiting technique showed selectivity with no colonization of other tested soil-borne pathogenic fungi (Table 2). In addition, cassava is colonized by other pathogen in quite low level when artificial infestation of pathogen at a density 10^3 cfu/g non sterilized soils, is only 3.75% (Table 3) compared to *R. microporus* (18.25%) (Table 2).

Table 2. Colonization of soil-borne fungi on cassava root bait in artificially inoculated soil.

Soil-borne Fungi	**Colonization rate (%) at inoculum density (cfu/g soil)**	
	10^3	**10^4**
Rigidoporus microporus	22.50 ± 3.25 b	56.25 ± 7.40 c
Rhizoctonia solani	0 a	0 a
Phytophthora palmivora	0 a	0 a
Fusarium oxysporum	0 a	0 a
Ganoderma boninense	0 a	0 a

Numbers followed by same symbols are not significantly different by DMRT test at P < 0.05

Table 3. Colonization of other fungi on tested cassava root bait.

Treatment	Colonization frequency (%) at different propagule density of *R. microporus* (cfu/g) soil			
	10^2	10^3	10^4	10^5
Sterilized soil	0 a	0 a	0 a	0 b*
Unsterilized soil	2.50 ± 0.35 b	3.75±0.35 b	0 a	0 b

*Numbers followed by the same letters are not significantly different by DMRT at $P < 0.05$

High selectivity of serological and molecular techniques have been used for the detection of *R microporus* from the soil (Dalimunthe et al. 2017; Oghenekaro 2016). The cassava root bait has the advantage of cheaper cost and needs less resource than the two methods. The optimum soil moisture for detection of *R. microporus* is 40% (Table 4). This in line with findings that determined soil moisture of 40 % is optimum soil moisture in the field for development of white root disease (Apriastika et al. 2015; Parasayu et al. 2016).

Table 4. Colonization of white root fungus, *Rigidoporus microporus*, on cassava rootbait as affected by the different soil water content.

Water content (%)	Colonization frequency (%)
20	15.00 ± 3.53 a
30	38.75 ± 4.15 b
40	85.00 ± 6.13 c
50	30.20 ± 3.54 b

Numbers followed by same letters are not significantly different by DMRT at $P < 0.05$

Comparison of cassava root bait technique and selective medium plating technique. Cassava root bait technique is comparable to other technique, selective medium plating technique. There is linear function between cassava root bait and selective medium plating technique with high coefficient of determination ($R^2 = 0.71$), P=0.0001 (Fig. 1). However, cassava root bait has the advantage over selective medium, in which the first requires less support of laboratory equipment, therefore more practical for farmers and agricultural officers to apply

Detection is important step for developing management action of plant pathogens.. *Rigidoporus microporus* or white root fungus (WRF) is one of destructive soilborne pathogens of para rubber plantation in the world (Nandris et al. 1988; Jayasinghe 2011), and causing death of hundreds thousands of trees in Indonesia (Situmorang and Budiman 2003). Inoculum source of the fungus come from alternate host, since it has wide range of host plants (Farid et al. 2009). Furthermore, even in absence of host plants, the fungus can survive in colonized residue of tree stumps, free mycelium or rhizomorph on plant debris in the soil, which will act as inoculum for next plantation (Nandris et al. 1988; Jayasinghe 2011). Therefore, the detection of inoculum in soil in plantations and nurseries is important to determine appropriate management.

Cassava root bait technique is a technique which is simple, needs less laboratory equipment, and relatively effective and cheap. Thus, farmers and extension workers can use this technique. The detection of *R. microporus* in soil is important because farmers use soil as a medium in polybags for growing seedlings. The detection of the fungus in the soil will allow timely diagnosis and management of the disease.

CONCLUSION

A practical technique, namely cassava root bait, for propagule detection of white root fungus, *Rigidoporus microporus*, from the soil was developed. It was proven to be an effective, selective and fairly sensitive technique for detection of WRF with a minimum propagule density of 10^3 cfu/g soil. The cassava root bait is selective and comparable with plating on selective Worrall media.

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