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Potential of Fungi Isolate as a Biological Control of White Root Disease (*Rigidoporus sp.*) on Rubber Plants

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ABSTRACT

Rigidoporus sp. is a white root fungus which is the main diseases problems on rubber plants in the wet and dry lands of South Kalimantan. An economically and ecologically beneficial control solution for this problem soil contagion was by using antagonistic fungi. This research was aimed to study the potential of *rhizosphere* and *endophytic* fungi in swampland, to control white root fungus on rubber plants. The research used exploratory method. *Rhizospheric* fungi were explored from swamp rubber plant roots at Pulau Damar village Hulu Sungai Utara District; *endophytic* fungi were isolated from leaves and jelutung swamps (*Dyera lowii*) which is rubber-like plants. The isolates found then were screened based on the percentage of inhibition using the dual culture method between pathogens and test isolates, also observed the interaction mechanism and viability test. The research in the laboratory was arranged using a Completely Randomized Design with one factor i.e. the type of antagonistic fungi. Five isolates which had the highest inhibitory effect were isolates I13K4R, I7K3R, I1K2R, I6K2R, and I1E with successive inhibitions 95.00%, 83.00%, 76.50%, 62.50% and 53.00%. Thus, isolates have included potential antagonistic agents because they had the ability in space competition more than 50% and proven to be able to paralyze *Rigidoporus*. The viability test results showed that the isolate that had germination capability of more than 60% were respectively I13K4R 88.05%, I7K3R 87.33%, I1K2R 86.93%, I6K2R 72.85%, and I1E 74.78%.

Keywords: antagonists, rubber-like plant, swampland, white root fungus

1. Introduction

South Kalimantan is one of the centre of rubber plantations in Indonesia. The use of swamps as rubber plantations is the key to success in developing rubber commodities. The area of rubber plantations originating from swamps in South Kalimantan in 2016 is 269,300 Ha and increased from year to year. Rubber productivity in South Kalimantan 1,047 kg sheets/year is below the national productivity of 1,500 kg sheets/year or only 63.85% of the potential productivity of rubber, this is due to the condition of plants that are partially old and damaged (30%) and attacked by fungi White Root Fungus what is called Jamur Akar Putih (JAP) in productive rubber plants. (Plantation Office, 2016). This disease is caused by the fungus *Rigidoporus microporous* (Abbot et al., 2014). Based on the prior observation taken place at the research location at Pulau Damar village, Banjar district, JAP attacked the rubber plantation as high as >60%. The JAP attack will get worse if not carefully opening the former forest (Semangun, 2008). This is because JAP can survive on plant debris in the soil.

Swamp Jelutung is a type of native plant that grows on peat swamps, with natural distribution areas on Sumatra, Peninsular Malaysia, and Kalimantan. This local tree can also produce a latex-like rubber. Jelutung sap has a high economic value, including being used as raw material for chewing gum, cosmetics and insulators (Coppen, 1995 in Waluyo et al., 2012).

Biological control using antagonistic fungi is a recommended way of protecting plants from soil-borne pathogens (Pal and Gardener, 2011). The ability of antagonistic fungi to supress soil-borne pathogens is by protecting plants from pathogenic infections. Antagonistic fungi indirectly inhibit pathogens by competing the space and nutrients. Biological agents can also directly fight the pathogens by hyper parasite mechanism and producing antibiotics (Kohl et al., 2019). Based on Benitez et al., (2004) antagonistic fungi such as *Trichoderma harzianum, T. viridae* and *T. virens* could control JAP pathogens until 90%. Amaria *et al., (2013)* reported that antagonistic fungi *T. harzianum, T. viridae* and *(Penicillium) lilacinus* were potential in suppressing JAP disease.

Biological activity in the soil is mostly concentrated in the topsoil, the depth of which can differ from a few cm to 30 cm. The use of swamps as rubber plantations results in a low microbial population in that area. This is because poor drainage treatment in swamps can affect the microbial state (Nielsen and Winding, 2002). Several studies have shown that antagonistic fungi are capable as biological control agents for JAP, but until now research on microbes from wetlands that can inhibit JAP has not been done. This study aimed to explore the antagonistic fungi in the wetlands that can inhibit JAP growth.

2. Materials and Methods

Materials

Rhizospheric fungi test isolates used came from soil and water in the *rhizosphere* of healthy rubber plants among rubber plants that were attacked by JAP. Location of isolate taking, in wetland rubber plantations (klon PB 260) in Damar Island Village, Hulu sungai utara District Regency of South Kalimantan. The endophytic isolate of the test was isolated from leaves and node of swamp jelutung (Dyera lowii) plants. This plant is a kind of rubber plant that lives in swamps. The research method used an explorative method. Activities continued in the laboratory which included a screening of exploratory isolates. This screening is based on the percentage of inhibition using the dual culture method between pathogens and test isolates. This research was arranged by using a completely randomized design with one factor, isolate antagonistic agents. The screening was based on the percentage of inhibition and five isolates were found with inhibitory effect > 50%.

The observations were also made on the mechanism of interaction between pathogens and tested isolates. The viability test of isolated fungi conidia was also observed through germination capacity. The percentage of inhibition and conidia viability were analysed in variance test and continued with the different test of the DMRT mean value.

Place and time

This research was conducted at the Phytopathology Laboratory, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru.

Exploration, Isolation and Purification

Rhizospheric fungi exploration was carried out by taking soil around the rhizosphere of healthy rubber plants around diseased plants, with a depth of 10-30 cm of soil taken with water. Isolation of *Rhizospheric* fungi from soil and water is carried out using a multilevel dilution method using PDA media (Nurhayati, 2010), then incubated at room temperature. Purification was carried out by separating the isolates that grew with different colony morphologies. Endophytic isolates were isolated from the leaves and swamp jelutung. The method used for endophytic isolation followed the Deepthi method et al. (2018).

In vitro Isolate screening

All isolates obtained were tested for their ability to inhibit *Rigidoporus* sp with the dual culture method (Rahman *et al.,* 1976). The calculation of the percentage of inhibition uses a formula developed by Skidmore and Dickinson (1976).

Mechanisms of Interaction Fungi and Pathogens.

The dual culture method was used to observe the interaction mechanism of space competition. The media cube method was used to observe the parasitic interaction mechanism by cutting PDA media and sterile scalpel with a size of 1x1 cm2, and then the pathogen hyphae were placed on the side of the

PDA media opposite the hyphae of test isolates. The culture was placed on microscope slide and covered with cover slip then observed the mechanism of interaction between the growth of pathogenic mycelia and tested isolates.

Conidia Viability Test

Viability is the conidia's ability to germinate. Viability test is done by taking PDA media using cork borer and placed on a sliding glass then dropping fungi suspension as much as one drop on the PDA media and cover with glass cover. The number of germinated fungi was counted. The calculation of conidium viability followed

-CV $= \frac{\sum GC}{\sum GC + \sum CNG} X\ 100\%$

Description:

CV = Conidium viability

$\sum$ GC = Germinated Conidia

$\sum$ CNG = Conidia that does not germinate

3. Results and Discussion

Results

The results of rhizospheric fungi exploration from swamps obtained 24 fungi isolates, consisting of 14 isolates from rubber rhizosphere and 10 isolates from swamp jelutung endophytes. Pathogens of JAP disease have been isolated from white rhizomorphs such as nets that spread to all parts of the affected root JAP. The results of pathogen isolation show pathogenic colonies. Microscopic observation of this fungus has a lot of hypha branching with septa and has a clamp connection

Screening test results

Screening test results based on inhibition test showed that the percentage of inhibition of isolates from rubber rhizosphere originating percentages ranged from 19.5% -95.0% and endophytic test isolates from swamp jelutong 16.5% -53.0%. Based on the percentage of inhibition, five test isolates were selected that have a percentage of inhibition> 50% i.e. isolate I13K4R shows the percentage of inhibition (95, 0%), I7K3 (83, 0%), I1K2R (76, 50%), I6K2R (62, 50%) and I1E (53, 00%).

The growth of pathogens that were grown together with the test isolates showed that the growth was suppressed by the test isolates reaching 95.0% in isolates I13K4R (Figure 3.). Between I6K2R and I1K2R and I7K3R test isolates showed no significant effect on *Rigidoporus* sp., but between I13K4R test isolates were significantly different from I1K2R, I6K2R, and I7K3R in inhibiting the growth of Rigidoporus sp.

Table 1.Percentage of inhibition of test isolates against White Root Fungus

No	isolate code	Inhibition (%)
1	I1E	53.00 a
2	I6K2R	62.50 ab
3	I7K3R	73.00 b
4	I1K2R	76.50 b
5	I13K4R	95.00 c

Description:

I1K2R: Isolate 1 garden 2 rhizosfer

I6K2R: Isolate 6 garden 2 rhizosfer

I7K3R: Isolat 7 garden 3 rhizosfer

I13K4R: Isolate 13 garden 4 rhizosfer

I1E: Isolate 1 endofit

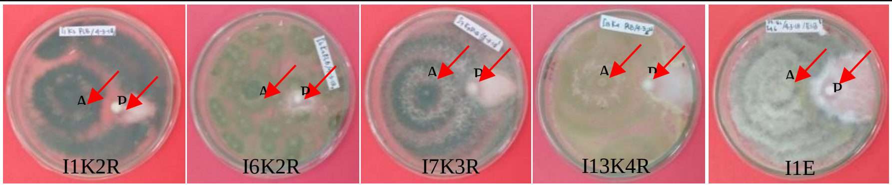
Interaction mechanism

The interaction mechanisms observed were space competition, antibiosis, and parasitism. In inhibiting the growth of JAP, the five test isolates showed the ability of the mechanism of interaction of space competition and parasitism, but in this observation, no antibiosis was found in the test isolates against pathogens (Table 1).

Table 2.Observation of interaction mechanisms

Isolate code	Interaction mechanism			Sample origin
	Competition	Antibiosis	Parasitism	
I1K2R	+	-	+	Rubber Rhizosphere
I6K2R	+	-	+	Rubber Rhizosphere
I7K3R	+	-	+	Rubber Rhizosphere
I13K4R	+	-	+	Rubber Rhizosphere
I1E	+	-	+	Jelutung endophytes

In the competition space, the growth of test isolates was more dominant than pathogens and the growth of pathogens was seen to be depressed due to the presence of test isolates (Figure 2.).



Description:
A: Antagonist P: Pathogen

Figure 2.Observation of space competition for 7 days

Conidium Viability

The viability test results showed the percentage of spore germination of five test isolates ranged from 72.85% -88.05% and there were no significant differences in each of the test isolates (Table 3)

Table 3.Test results of mean values for the viability test

No	Isolate code	Percentage of average germination (%)
1	I6K2R	72.85 a
2	I1E	74.78 ab
3	I1K2R	86.93 b
4	I7K3R	87.33 b
5	I13K4R	88.05 b

Discussion

Microscopic observations of these pathogenic fungi have hyaline hyphae, have septa, there are clamp connections and have many hyphal branches. The results of microscopic observations in this study are in line with research by Farhana et al., (2017), Shofiana et al., (2015), Novianti et al., (2011), Jayasinghe and Wettasinghe (1998) in Jayasuriya and Thennakoon (2007) which states that *Rigidoporus lignosus* has hyphae that are hyalinous, encircling hyphae and has a clamp connection but is different from the study of Kaewchai and Soytong (2010) which states the absence of a clamp connection (clamp connection) on *Rigidoporus microporous*. Based on the colony and microscopic morphology tested in this study, the pathogenic fungus used was Rigidoporus sp.

The results of fungi exploration from wetlands obtained 14 isolates. Rhizosphere fungi are more common than endophytic fungi, 14 isolates of rhizosfer fungi and 10 endophytic fungi isolates. This is in line with the research of Oku et al., (2012) that the presence of *rhizosphere* fungi is more abundant than endophytic fungi residing in plant tissues. Pathogen suppression is influenced by heterotrophic and pathogenic responses of microorganisms to drought and waterlogged conditions and their ability to recover. A wider niche for microorganisms results in a higher chance of surviving extreme conditions and, consequently, a higher chance of being present in the recovery phase. (Van Agtmaal *et al*., 2015).

The results of screening tests between test isolates and pathogens showed that five test isolates had a significant inhibitory effect on JAP pathogen isolates with a percentage value of inhibition >50%. The highest percentage of inhibition is owned by isolates I13K4R (95, 00%) and lower I1E (53, 00%). Based on Ubogu (2013) some antagonists which have inhibition of more than 50% then the antagonist agent can be used as a controlling agent. Mariana *et al.,* (2016) also stated that in vitro the antagonistic agent, the fungus Trichoderma sp. can control JAP (*R. lignosus*), because Trichoderma sp. is a strong competitor in suppressing the growth of pathogenic fungi.

During the seven days of observations, space competition between test isolates and pathogenic fungi was observed (Table 1). According to Heydari and Pessarakli (2010), space competition occurs because of competition in place and nutrition in growth media. In nature, nutrients come from the soil and the *rhizosphere*, but in limited quantities so to get it you have to compete with other microbes including pathogens. Therefore antagonistic fungi with strong competition potential have the potential to be used as biological control agents. Liu et al (2013) prove that nutritional competition between the antagonist *Kloeckera apiculata* and *Penicillium italicum* causes blue mold in oranges. The results of the study indicate that K. apiculata is a competitor who wins competing with pathogens to increase its effectiveness as a biological control agent. In inhibiting the growth of pathogenic fungi, antagonistic fungal agents use sugars and carbohydrates available as precursors of secondary metabolites in inhibiting the formation of pathogenic fungal spores while in mycopantoitism Soesanto (2008) explains that mycelium antagonists that grow toward the thogenic mycelium are attracted by the presence of α-lectin-bound proteins. With chitin which is used as a constituent of cell walls. In the attachment stage, hyphae antagonists penetrate/break the pathogen cell wall by producing chitinase, glucanase, protease, and xylanase enzymes to decompose the pathogen cell wall. In mycoparasitism test, all visible hyphae were isolated from pathogenic hyphae and surrounded it like forming a coil. According to Kullnig et al., (2000) and Gupta et al., (2014) in paralyzing pathogenic hyphae, antagonistic agents have chitinase and protease compounds so that they are successful in the process of penetrating pathogen cells

Spore viability for 24 hours showed that the viability was not significantly different between test isolates, with a percentage of germination> 60% in the range of 72.85% -88.05% (Table 2). According to Chen and Ginette (2002), the viability of a fungus spore is influenced by the success of the spore's sprouts. According to DPP (2014) that viability meets the requirements if the percentage of germination> 60% and thus all test isolates have viability as a supportive agent for antagonists.

4. Conclusions

Rhizospheric fungi were explored from swamp rubber plant obtained 14 isolates from rubber rhizosphere and 10 endophytic isolates from jelutung swamps and among them five isolate of I13K4R, I7K3R, I1K2R, I6K2R, and I1E inhibited mycelial growth of *Rigidoporus* sp more than 70%, conidial germination more than 70%, space competition and parasitism. They are suggested as potential biocontrol agent *Rigidoporus*.

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