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Efficacy test of biological agent *Trichoderma* spp. against white root fungus disease (*Rigidoporus microporus*) in rubber tree (*Hevea brasiliensis*)

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ABSTRACT

White root rot disease caused by *Rigidoporus microporus* is one of the most destructive root diseases in rubber plantations, leading to significant yield losses each year. The study aimed to evaluate the efficacy of the biological control agent *Trichoderma* spp. in suppressing the development of white root fungus on rubber seedlings. Laboratory assays using the dual culture method and greenhouse trials with preventive and curative applications were conducted, involving treatments with *Trichoderma*-based biofungicide, chemical fungicide, and control. Laboratory results showed that *Trichoderma* spp. inhibited the growth of *R. microporus* by more than 44% on the fifth day after inoculation. In greenhouse experiments, preventive application of *Trichoderma* spp. was more effective than curative application, as indicated by improvements in stem diameter, plant height, and the number of leaves. Besides antagonistic activity, *Trichoderma* spp. contributed to improved soil fertility and plant growth. The lowest disease intensity (25.2%) was observed in the preventive treatment with *Trichoderma*, while in curative treatments, its effectiveness was comparable to that of chemical fungicides. The findings confirm that *Trichoderma*-based biofungicides are effective in controlling white root rot disease while simultaneously enhancing plant growth, making them a sustainable alternative to chemical fungicides.

Keywords: antagonist, biofungicide, biocontrol, disease control, plantation

INTRODUCTION

Rubber trees (*Hevea brasiliensis*) are widely cultivated across tropical regions of Asia, including Indonesia, and remain the primary global source of natural rubber. This commodity plays a crucial role in Indonesia's economy, particularly in Sumatra and Kalimantan, where rubber cultivation is dominated by smallholder plantations (Otten et al., 2020). However, national rubber productivity faces serious bioecological challenges, especially white root disease (WRF), caused by *Rigidoporus microporus* (Amaria et al., 2024). This disease leads to significant economic losses (Andrew et al., 2021) and is considered the most destructive disease of rubber worldwide (Go et al., 2021). According to Saidi et al. (2023), WRF is widespread in major rubber-producing regions such as Malaysia, Thailand, Sri Lanka, Indonesia, the Philippines, and several West African countries including Nigeria. It damages scion gardens, young non-productive plants, and often causes severe decline within two to four years (Ainusyifa et al., 2024).

In Indonesia, WRF reduces rubber production by 5–15% annually, with incidences reaching 30% in plantations around three years old and up to 40–60% in smallholder farms. In West Kalimantan, the disease affected 10,000 ha out of 500,000 ha in 2003, causing losses of about 3 million USD (Mohammed et al., 2014). In Asahan Regency, WRF infestation in 2014 reached 361.17 ha, with an estimated yield loss of 216.7 ha every three months, resulting in total losses of 1,159,671,750 rupiah or 8.53% (Rahayu

et al., 2017). Nationally, plant mortality from WRF leads to annual financial losses of around 1.8 trillion IDR or 200 million USD (Situmorang et al., 2007). In Malaysia, the disease reduces yields in older plantations by up to 50% (Go et al., 2023). Symptoms include root decay, brown discoloration, white mycelium or rhizomorphs, leaf yellowing, defoliation, inward leaf curling, and branch dieback (Maiden et al., 2022; Dalimunthe et al., 2023).

Globally, WRF has been managed using technical, physical, chemical, and biological approaches. In Malaysia and Thailand, planting tolerant rubber clones alongside eradication of infected trees has effectively reduced disease spread in large plantations (Go et al., 2021; Wattanasilakorn et al., 2017). Chemically, fungicides such as propiconazole or mixtures containing hexaconazole are effective, but they are expensive, environmentally risky, and may promote pathogen resistance (Olaniyi & Szulczyk, 2022). In Lampung, physical control using ground cover plants like *Alpinia galanga*, *Sansevieria auranthii*, and *Maranta arundinacea*, combined with *Trichoderma* spp. and natural sulfur, has significantly decreased WRF incidence. Biological control using biofungicides is increasingly recognized as an environmentally friendly alternative (Ghorbanpour et al., 2018). These antagonistic microorganisms suppress pathogens through mechanisms such as parasitism, antibiotic production, nutrient competition, enzymatic degradation, and induction of plant defenses, while also reducing dependence on chemical pesticides that pose health and ecological concerns (Essiedu et al., 2020).

One of the most effective biological control agents against WRF is *Trichoderma* spp., a fungus widely recognized for its strong antagonistic activity (Firmansyah et al., 2024). Trichoderma suppresses *R. microporus* through competition, mycoparasitism, and the secretion of hydrolytic enzymes such as chitinases and cellulases. It also strengthens plant defenses by increasing the activity of defense-related enzymes and producing antifungal secondary metabolites (Vinale & Sivasithamparam, 2020). Shabrin et al. (2025) reported that biofungicides containing Trichoderma strains offer an effective and environmentally friendly method for managing root diseases. Four species *T. asperellum*, *T. koningiopsis*, *T. spirale*, and *T. reesei* suppressed *R. microporus* growth by more than 75% in dual-culture assays. Non-volatile filtrates of *T. asperellum* at 50–75% concentrations inhibited pathogen growth by up to 78.80%, while volatile compounds produced as much as 55% inhibition. The purpose of this research was to evaluate the effectiveness of *Trichoderma* spp. in suppressing WRF in rubber plants, particularly because its application remains limited in South Sumatra.

MATERIALS AND METHODS

Preparation

The research was conducted at the Protection and Greenhouse Laboratory at Pusat Penelitian Karet Sembawa, Banyuasin Regency, South Sumatra. The testing was conducted over a period of 6 months, starting in September 2020 and ending in March 2021.

Implementation

Inoculum Preparation

To obtain an isolate of *R. microporus*, root sections infected with white root rot fungus were taken from the field. The root sections were isolated on PDA medium and then purified after the fungal mycelia grew. Using a sterile cork borer, 0.5 cm in diameter and 1–2 mm thick, fungal plate inocula were made to rejuvenate the pure *R. microporus* culture. This inoculum was then inoculated right in the middle of the medium (Dalimunthe et al., 2019).

Population Size and Antagonistic Tests

The population count was obtained by performing dilutions up to 10^{-3}, isolating the dilution results on PDA and MA/NB media, incubating, and counting the number of colonies that grew after the second day. The antagonism test between antagonistic microorganisms and *R. microporus* as a pathogen was conducted using the dual culture method on Petri dishes containing PDA medium. Each treatment consisted of 10 Petri dish replicates, with a Petri dish diameter of 9 cm. The antagonistic microorganism inoculum was inoculated onto the

medium 3 cm from the edge of the dish, while the *R. microporus* fungal inoculum was placed 3 cm from the edge of the Petri dish on the opposite side (Go et al., 2023) (Figure 1). As a control, *R. microporus* was inoculated without the addition of antagonistic microorganisms. Observations of antagonistic interactions were made daily until seven days after inoculation.

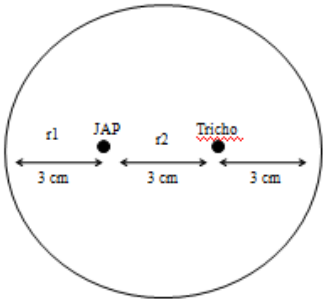

Figure 1. Antagonistic test method

$$\text{Percentage of Inhibition} = \frac{r1 - r2}{r1} \text{ X } 100\%$$

Description:

r1 = Radius of the pathogenic colony away from the antagonistic fungus

r2 = Radius of the pathogenic colony approaching the antagonistic fungus

Greenhouse Testing

For greenhouse testing, two main factors were used: preventive and curative, with three treatments as sub-factors: a) Trichoderma product, b) Commercially available chemical fungicide, c) Control (no treatment). Each treatment consists of 10 plants, so a total of 180 plants were needed, with the following breakdown:

2 factors (preventive and curative) x 3 treatments/factors x 3 replications/treatment x 10 plants/treatment = 180 sample plants. Stages of greenhouse testing activities:

A. Preventive

1. Mix the soil in each polybag with 100 g of *Trichoderma* spp., plant 1 rubber seedling or stump, let it colonize, and maintain soil moisture.
2. After being colonized by Trichoderma, place the foodbase into a polybag, observe the attack and severity of the disease caused by JAP by scoring from before planting the foodbase until the end of the study.

B. Curative

1. Prepare the polybag seedlings.
2. Input the foodbase, observe the development and severity of the disease caused by WRF by scoring, starting from before planting the foodbase until the end of the study.
3. After seeing WRF mycelia at disease stage 1, sow *Trichoderma* spp. at a rate of 50 g, repeating the application 3 times at 3-week intervals.
4. Conduct observations using the observation parameters.
5. For chemical fungicide treatment, water the root area of the plants with a dosage of 5 cc/liter of water, with each plant receiving only 100 cc of water from the mixed suspension.

Calculation of WRF Disease Intensity

The development of WRF was observed by opening the polybags and examining the root systems of the rubber seedlings three months after treatment. The effectiveness of the biofungicide formula was determined based on the JAP attack scale according to (Go et al., 2021) as followed:

Table 1. Scale of WRF disease attack intensity

Scale	Description
0	No WRF mycelium was attached to the seedling roots;
1	WRF mycelium was attached to the seedling roots.
2	WRF mycelium was found attached to the seedling roots and had penetrated into the tissues.
3	The tissue inside the root was black and rotting.
4	The plant was dead and the roots are rotten.

Next, the intensity of the disease attack was determined by the formula according to (Maiden et al., 2022) as followed:

$$IP = \frac{\sum_{i=1}^{n} n.v}{Z.N} x100\%$$

With

IP : disease intensity;

n : number of scaled plants v;

v : scale i; and

Z : highest scale value

N : number of plants observed

Data Analysis

Attack scale data were analyzed using one-way ANOVA and further tested using the

Duncan Multiple Range Test in the Statistical Analysis System (SAS) program (Kusdiana et al., 2015).

RESULTS

Laboratory Testing

The results of the spore concentration test showed that there were 7,5 x 10^6 Trichoderma spores per gram. The colonies of *R. microporus* and *Trichoderma* spp. connected mycelia on the second day after *Trichoderma* spp. was isolated as an antagonistic fungus. On the fifth day after the antagonistic isolation, the development of *R. microporus* stopped. The opposing fungal aerial hyphae began to break down, and the *R. microporus* colonies turned yellowish at the contact area. The colonies were initially white but later turned yellowish over the same area (Figure 2). Because *Trichoderma* spp. produces a number of enzymes that break down cell walls and stop the growth and activity of white root rot fungi, the hyphae of *Trichoderma* spp. grow on the hyphae of R. microporus and undergo lysis.

Figure 2. Antagonistic test of *Rigidoporus. microsporus* and *Trichoderma* sp.

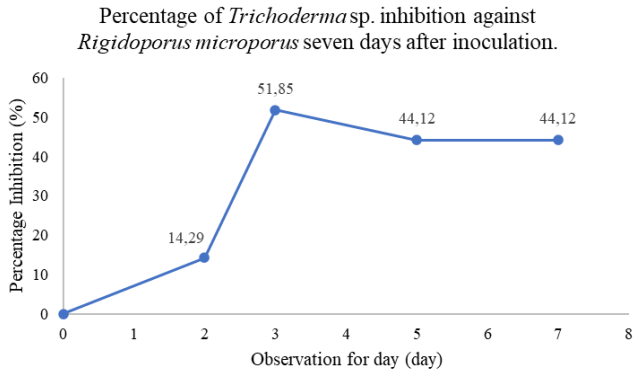

Figure 3. Percentage of *Trichoderma* sp. inhibition against *Rigidoporus microporus* seven days after inoculation

Laboratory testing results showed that the biological agent *Trichoderma* spp. could easily suppress white root fungus. On the fifth day of white root fungus isolate (*R. microporus*) growth, the percentage of inhibition on day 3 reached 51.85% and on day 7 decreased to 44.12%. (Figure 3). This was because the product contains *Trichoderma* spp, a fungus that could suppress the growth of *R. microporus*.

Greenhouse Testing

Greenhouse test results show that using *Trichoderma* spp. preventatively yields better results than using it curatively against the stem diameter. The application of *Trichoderma* spp. Before the white root fungus attack was a preventive measure, as shown, while a curative measure was its application after the white root fungus attack had occurred. *Trichoderma* spp. also has additional effects; not only does it suppress the development of white root fungus, but *Trichoderma* also had several other influences, namely helping plant growth, such as increasing stem diameter, plant height, and the number of umbrellas on polybag seedlings (Table 2). This was because *Trichoderma* spp. has the ability to decompose organic compounds, which can affect the physical properties of the soil and the availability of nitrogen in the soil. Table 3 also shows that plant height has a significant effect on both preventive and curative applications. Nitrogen conditions in the soil greatly affect plant height. This causes the plants to grow well and affects their growth and production.

Table 2. Results of the antagonistic test of biological agents against *Rigidoporus microporus* in the greenhouse

Parameter	Single Factor		Interaction Factor
	Preventive	Curative	(Preventive x Curative)
Stem Diameter	√	–	–
Plant Height	√	√	–
Number of Shades	–	√	–
Intensity of white root rot	–	–	√

Description: √ = has a real impact, - = It has no impact

Among the preventive treatments, control treatment (P3K1) had the highest attack intensity of 50.5% and the lowest attack intensity of 25.2% with *Trichoderma* (P1K1). On the other hand, among the curative treatments, P3K2 treatment

had the highest attack intensity of 50.45% and the lowest attack intensity of 20.8% with chemical fungicide (P2K2). The intensity of white root fungus (WRF) showed a significant effect on both preventive and curative interactions (Table 1 & Table 2). This indicates that the product should be applied simultaneously, both preventively (before WRF attack) and curatively (after WRF attack). Preventively, *Trichoderma* sp functions as a soil organism that will colonize the soil quickly before other fungi like *R. microporus* attack. Conversely, curatively, *Trichoderma* sp. will function as a biological agent that could control/suppress *R. microporus* attacks.

Table 3. Statistical analysis results of *Trichoderma* spp. application on the intensity of white root fungus attack (%)

Treatment	Month			
	1	2	3	4
P1K1	25.0a	25.8d	20.0c	25.2c
P1K2	25.0a	40.5b	38.8d	28.4b
P2K1	25.0a	36.7c	32.6d	30.2b
P2K2	25.0a	20.5d	20.8bc	20.8bc
P3K1	25.0a	50.0a	50.4a	50.5a
P3K2	25.0a	50.0a	50.2a	50.4a

Note: P1= *Trichoderma* spp., P2 = Chemical fungicide, P3 = control, K1 = Preventive, K2 = kurative

DISCUSSION

The research results show that the application of *Trichoderma* spp. is able to halt the development of *Rigidoporus microporus* by more than 44% on the fifth day after inoculation. Direct contact between hyphae, changes in the shape of pathogenic hyphae, and the lysis process caused by the activity of hydrolytic enzymes from *Trichoderma* sp., such as chitinase and β-1,3-glucanase, are evidence of the antagonism mechanism. The change in color of the pathogen colonies from white to yellowish in the contact area indicates that the pathogen cell walls have been degraded. According to the research by Tyśkiewicz et al. (2022), the mechanism of antagonism by *Trichoderma* spp. occurs thru direct physical interaction. Trichoderma penetrates the hyphae of the pathogen, and then the pathogen's cell wall undergoes local lysis due to hydrolytic enzymes such as chitinase, β-(1,3)-glucanase, and protease. This changes the color of the pathogen colony. Greenhouse trials also showed that preventive use was more effective than curative use; preventive use increased stem diameter, plant height, and the number of umbrellas on rubber seedlings. Comite et al. (2021) stated that *Trichoderma* spp. and *Azotobacter* sp. significantly increased plant biomass, both fresh and dry weight, compared to a single treatment or control. This indicates that these two organisms work together to promote plant growth.

Trichoderma produces secondary metabolites and hydrolytic enzymes that help stop the growth of soil pathogens like *Rigidoporus* spp. (Khan et al., 2023). Trichoderma strengthens plant resistance to pathogens and functions as a biocontrol agent. Manzar et al. (2022) stated that *Trichoderma* spp. combat plant diseases thru various antagonistic strategies. These strategies include antibiosis, mycoparasitism, competition for nutrients and space, plant growth stimulation, and induced plant defense mechanisms. The use of Trichoderma spp. not only suppresses the intensity of *R. microporus* attacks but also has a positive effect on vegetative growth. Compared to the use of chemical fungicides, which are indeed effective curatively, the findings of this research indicate that Trichoderma is not significantly different from chemical fungicide application in terms of curative effect, but Trichoderma has the added advantage of improving soil fertility. According to Enshasy et al. (2020), Trichoderma is a saprophytic fungus commonly found in places such as forest soil, roots, and leaves. This fungus is categorized as a soil fungus due to its rapid growth. Trichoderma can also utilize various types of complex substrates and function as strong protection against various toxic chemicals, as well as enrich the soil. This is primarily due to heterotrophic interactions such as opportunistic endophytism.

The effectiveness of using *Trichoderma* spp. fungi in controlling white root fungal disease have long been known. *Trichoderma* spp., known as a cosmopolitan species, can thrive in root, soil, and leaf environments and has several advantages as a plant pathogen control agent. Trichoderma spp. produce hydrophobins, a type of cysteine-rich protein that functions to enhance plant growth and resistance. This compound stimulates root growth and has the ability to alter plant

physiological changes, making plants more resistant to various pathogens (Pandey et al., 2021). The application of antagonistic agents can directly suppress pathogen inoculum, stop pathogen colonization, and protect plant seeds and root growth from infection. Antagonistic agents can also inhibit pathogens through antibiotic secretion, competition for space and/or nutrients, inducing plant resistance, and direct interaction with pathogens. The interactions that occur are hyperparasitic, hyperpathogenic, or predatory, involving the destruction of propagative units (propagules) or biomass, thereby reducing inoculum density and pathogen activity (Swain & Mukherjee, 2020). Due to differences in morphology and physiology, each species of *Trichoderma* spp. has varying abilities to control pathogenic fungi. Some species of *Trichoderma* sp. function as biological control agents. Some of these include *T. atroviride, T. harzianum, T. asperellum*, *T. virens, T. longibrachiatum,* and *T. viride*, all of which can be found on various cultivated plants (Guzmán et al., 2023).

CONCLUSION

The results of this study indicate that the application of *Trichoderma* spp. Based biofungicides is able to suppress the growth of *Rigidoporus microporus* by more than 44% in in vitro tests, with mechanisms of antagonism including direct contact between hyphae, morphological changes in the pathogen, and lysis due to the activity of hydrolytic enzymes. Greenhouse trials show that preventive application is more effective than curative in suppressing the intensity of white root fungal disease while simultaneously promoting the vegetative growth of rubber seedlings, such as stem diameter, plant height, and number of umbrellas. Besides functioning as a biocontrol agent, *Trichoderma* spp. can also improve soil fertility, thus providing a dual effect of plant protection and increased biomass. Thus, *Trichoderma* spp. has great potential to be used as an environmentally friendly alternative to replace chemical fungicides in the white root rot disease control strategy for rubber plants.

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