ANTAGONISTIC TEST OF RUBBER PLANT RHIZOSPHERE FUNGAL ISOLATES AT PT. BRIDGESTONE KALIMANTAN PLANTATION (BSKP) AGAINST *Pestalotiopsis* sp. IN VITRO

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ABSTRACT

Rubber plants (Hevea brasiliensis) are plantation commodities that play an important role in the Indonesian economy and are the main foreign exchange earner in the plantation sector. In the rubber company Bridgestone Kalimantan Plantation (BSKP), leaf fall disease is caused by the pathogen Pestalotiopsis sp. Control techniques using excessive chemical pesticides can damage the balance of the ecosystem and pollute the environment. One environmentally friendly alternative control is antagonistic fungi that can suppress the growth of pathogens that cause disease. The purpose of this study was to determine the ability of antagonistic fungi from the rhizosphere of rubber plants at PT BSKP to inhibit Pestalotiopsis sp. This study was conducted at the Phytopathology Laboratory of Lambung Mangkurat University. Sampling was carried out at the rubber plantation of PT BSKP, Tanah Laut Regency, South Kalimantan. Samples were taken from 4 plots where and each plot consisted of 5 subplots (5000 m2). From each subplot, 1 point/tree was taken. The method was a Completely Randomized Design (CRD) with 8 treatments (antagonistic isolates) and 3 replications. The results of the isolation of rubber plant rhizosphere fungi obtained 15 pure isolates which were then selected by pathogenicity test and received 7 pathogenic isolates and 8 non-pathogenic isolates. Furthermore, an inhibition test was carried out to determine the ability of antagonistic fungi, the results of this test contained 2 isolates, namely B3T7 and B2T1 which had an inhibition percentage of >50%, respectively, 66.01% and 51.78%. In addition, isolate B1T2 with an inhibition percentage of 29.39% has an antibiosis mechanism that can also be categorized as an antagonist. Further identification by morphological observation showed that isolate B3T7 was Trichoderma sp., isolate B2T1 was Purpureocillium sp. and isolate B1T2 was Penicillium sp.

Keywords: Antagonistic Fungi, Hevea brasiliensis, Pestalotiopsis sp., PT BSKP.

1. INTRODUCTION

Indonesia once dominated world rubber production by outperforming other countries and has a great opportunity to exploit the market potential with world rubber demand that continues to increase from year to year. According to BPS (2022), Indonesia's dry rubber production in 2022 reached 2,717.08 thousand tons, a decrease compared to production in 2021 which reached 3.045 million tons. The decline in rubber production was partly caused by leaffall disease. According to Nikmah (2017), the loss caused by the pathogen that causes rubber leaf fall is a decrease in production of 25-30%. According to Febbiyanti & Fairuza (2020), the pathogen that causes leaf fall in rubber plants is *Pestalotiopsis* sp.
One of the agro-industries engaged in rubber processing in the form of RRS (Ribbed Smoked Sheet) is PT Bridgestone Kalimantan Plantation (BSKP). The results of Jannah's (2024) study stated that in the BSKP company, rubber leaf fall disease was detected caused by the *Pestalotiosis* sp. pathogen. The control technique that has been used so far is chemical. Excessive use of chemicals (pesticides) can damage the balance of the

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ecosystem. Therefore, an alternative biological control is used by utilizing antagonistic agents that can help suppress pathogens that cause disease. According to Zamrodah (2015), antagonistic agents have benefits that are directly related to residue-free agricultural production and support environmentally friendly agriculture in the global era. Microorganisms that act as antagonistic agents already exist naturally in the environment and have sustainable properties. These sustainable properties are proven by their ability to reproduce and reproduce naturally. Therefore, the author will conduct research on the inhibition test of antagonistic fungi from the rhizosphere of rubber plants at PT. BSKP to suppress pathogens that cause rubber leaf fall (*Pestalotiopsis* sp.).

2. RESEARCH METHODOLOGY

This research was conducted at PT. BSKP and the Phytopathology Laboratory of Lambung Mangkurat University. Sampling was carried out at the rubber plantation of PT. BSKP, Tanah Laut Regency, South Kalimantan. Samples were taken from 4 plots and each plot consisted of 5 subplots with an area of 5000 m2. From each subplot, 1 point/tree was taken. Samples were composited based on their respective plots so that 4 samples were obtained. Furthermore, 10 grams of soil was taken to be isolated on PDA media. This research was conducted using the Completely Randomized Design (CRD) method consisting of eight treatments of antagonistic fungal isolates. Each treatment was repeated three times so that there were 24 experimental units.

3. LITERATURE REVIEW

Pathogens Causing Rubber Leaf Fall Disease (Pestalotiopsis sp.)

The *Pestalotiopsis* sp. pathogen can produce several compounds that can disrupt the physiological processes in rubber plants. Leaves attacked by fungi will experience discoloration and brown spots which are thought to be caused by toxic compounds released by the pathogen. The spots continue to widen to a size of 1-2 cm, then the tissue around the spots undergoes necrosis. In severe attacks, it will cause leaves to fall continuously until the crown of the plant is bare.

4. RESULTS AND DISCUSSION

Isolation of Rubber Plant Rhizosphere Fungi at BSKP

Fungal isolation was carried out by multilevel dilution, namely 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. The isolated isolates were then purified. According to Kartika et al., (2012), purification was carried out to obtain pure isolates from an isolate that had been mixed with other isolates. The characteristics of the pure isolates were then observed, which can be seen in Table 1.

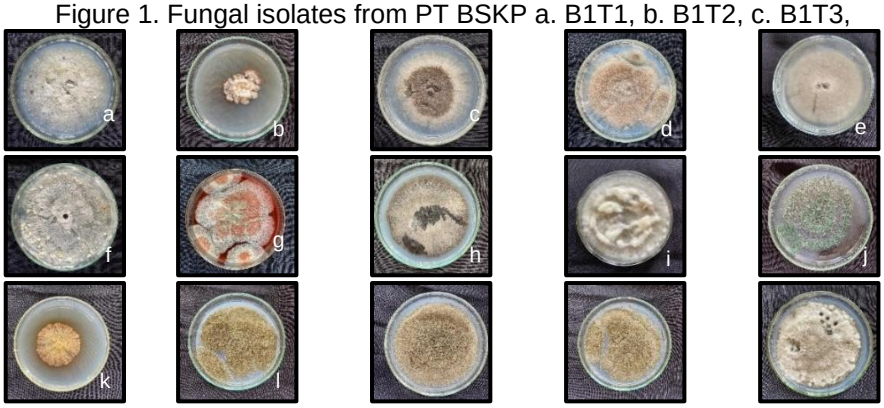
Table 1. Characteristics of isolated fungi

No	Isolate	Color	Shapes	Surface	Edge
1	B1T1	Yellowish white	Irregular round, ringed	Flat	Wavy
2	B1T2	Brownish white	Irregular round	Convex	Wavy
3	BIT3	Grayish white	Regular round	Flat	Flat
4	B2T1	Grayish pinkish purple	Regular round, ringed	Convex	Wavy
5	B3T1	Grayish white	Regular round, ringed	Flat	Flat
6	B3T2	Yellowish white	Regular round	Flat	Flat
7	B3T3	Red-green yellowish	Irregular round	Flat	Wavy
8	B3T5	Grayish green	Regular round	Flat	Serrated
9	B3T6	Black White	Regular round	Convex	Flat

Table 1. Continued

No	Isolate	Color	Shapes	Surface	Edge
10	B3T7	Green white	Round ringed	Flat	Flat
11	B4T1	Orange-brown	Irregular round	Flat	Serrated
12	B4T2	Green white	Round ringed	Flat	Wavy
13	B4T3	Yellowish green	Irregular round, ringed	Convex	Flat
14	B4T4	Green white	Round ringed	Flat	Wavy
15	B4T5	Green white	Round ringed	Convex	Flat

Figure 1. Fungal isolates from PT BSKP a. B1T1, b. B1T2, c. B1T3,



d. B2T1, e. B3T1, f. B3T2, g. B3T3, h. B3T5, i. B3T6, j. B3T7,
k. B4T1, l. B4T2, m. B4T3, n. B4T4, o. B4T5

Isolates from the purification of rubber rhizosphere at PT. BSKP has very diverse characteristics. According to Kennedy (2005), the rhizosphere area can increase the number and activity of microorganisms and become a location for interactions between microorganisms and plant roots.

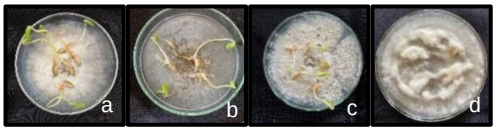
Pathogenicity Test

Fungal pathogenicity testing aims to determine whether the fungus is pathogenic or non-pathogenic. The test was carried out using cucumber seeds on each isolate consisting of 15 isolates. The test results showed 7 isolates were pathogenic and 8 non-pathogenic isolates.

Table 2. Results of the pathogenicity test

No	Isolate	Pathogenicity reaction
1	B1T1	+
2	B1T2	-
3	B1T3	+
4	B2T1	-
5	B3T1	-
6	B3T2	+
7	B3T3	-
8	B3T5	-
9	B3T6	+
10	B3T7	-
11	B4T1	-
12	B4T2	+
13	B4T3	+
14	B4T4	+
15	B4T5	-

Note: (-) = non-pathogenic, (+) = pathogenic



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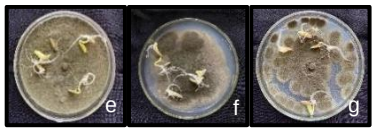


Figure 2. Results of pathogenicity test with cucumber seeds (pathogen)
a. B1T1, b. B1T3, c. B3T2, d. B3T6, e. B4T2, f. B4T3, g. B4T4

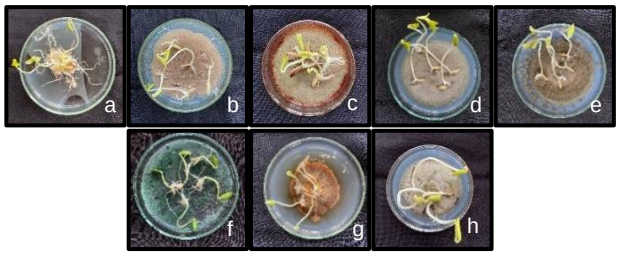


Figure 3. Results of pathogenicity test with cucumber seeds (non-pathogenic)
a. B1T2, b. B2T1, c. B3T1, d. B3T3, e. B3T5, f. B3T7, g. B4T1, h. B4T5

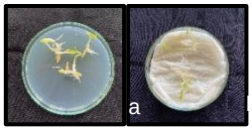


Figure 4. Results of pathogenicity test with cucumber seeds (control)
a. Control with Potato Dextrose Agar, b. Control with wet tissue

The test results in Figure 3, namely isolates B1T1, B1T3, B3T2, B3T6, B4T2, B4T3, and B4T4 showed abnormal sprout growth, rotting, and death, even the sprouts were completely covered by pathogenic fungal mycelium. According to Mulyani et al., (2023), seed pathogens can cause seeds to fail to germinate, cause rotting, inhibit growth, and cause sprout death. These symptoms occur because several types of fungi are known to produce secondary metabolites that are toxic to seeds and sprouts. Meanwhile, the results of observations of non-pathogenic isolates (Figure 4), namely isolates BIT2, B2T1, B3T1, B3T3, B3T5, B3T7, B4T1, and B4T5 showed normal growth, and even better than the control, especially in terms of root, stem, and flower bud growth. Non-pathogenic fungi can trigger germination by producing growth-regulating compounds. According to (Nassar et al., 2005) growth-stimulating hormones are divided into 5, namely abscisic acid, auxin, cytokinin, gibberellin, and ethylene. Furthermore, according to Campbell et al., (2008) there are types of fungi that can form mutualistic relationships with plant roots and play a role in fertilizing plants.

***Antagonistic Test Against Pestalotiopsis* sp.**

Non-pathogenic fungi detected from the results of the pathogenicity test were then continued with antagonistic tests against the pathogen *Pestalotiopsis* sp. with the dual culture method (Ningsih *et al.,* 2016). According to Anwar et al., (2018) this test aims to determine the ability of antagonistic fungal isolates to suppress pathogens that cause plant diseases

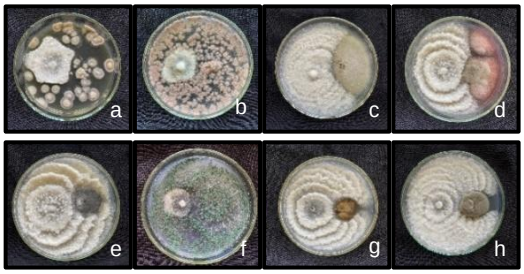
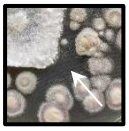


Figure 5. Antagonist test results a. B1T2, b. B2T1, c. B3T1, d. B3T3, e. B3T5
f. B4T7, g. B4T1, h. B4T5

Table 3. Inhibition rates & Mechanism of antagonistic fungi against *Pestalotiopsis* sp.

Isolate	Percentage of Inhibitory Power (%)	Mechanism			Picture
		Antibiosis	Competition	Overgrowth	
B1T2	29.39[b]	+	-	-	
B2T1	51.78[c]	-	+	-	
B3T1	7.11 [a]	-	-	-	-
B3T3	8.09[a]	-	-	-	-
B3T5	5.51[a]	-	-	-	-
B3T7	66.01[c]	-	+	+	
B4T1	27.78[b]	-	-	-	-
B4T5	9.80[a]	-	-	-	-

The test results showed that only 2 fungal isolates produced an inhibition percentage of >50% with the codes B2T1 and B3T7. Isolate B3T7 had the highest inhibition percentage of 66.01% and had an interaction mechanism in the form of competition and antibiosis. Isolate B2T1 had the second-highest inhibition percentage of 51.78% with an interaction mechanism in the form of space competition. In addition, there was an isolate with the code B1T2 which showed the highest antibiosis mechanism compared to other fungi. According to Melysa et al., (2013), antagonistic properties are interactions resulting from competition between two types of fungi (antagonist and pathogen) that grow side by side in one petri dish. This competition occurs because both fungi have the same needs, namely growing space and nutrients from the media used for growth. According to Chair et al., (2023), the antibiosis mechanism can be characterized by the formation of a clear zone between antagonistic fungi and pathogenic fungi in a space that is not overgrown by both fungi. The antibiosis mechanism that produces a clear zone is due to the presence of secondary metabolite compounds produced by fungi. Furthermore, according to Zivkonic et al., (2010) in the competition mechanism, antagonistic fungi will get more space and nutrients compared to pathogens so that the growth of pathogens is inhibited.

Morphological Identification

According to Hutabalian et al., (2015) antagonistic fungi that have inhibition power >50% have the potential to be antagonistic agents. In addition, according to Hudi (2014) if fungi can inhibit pathogens with one or more inhibition mechanisms, then the fungi can be said to be antagonistic. Therefore, fungi that have inhibition power >50% and fungi that have inhibition mechanisms against pathogens are then identified microscopically. The identified fungi have isolate codes B2T1, B3T7 and B1T2.

Table 4. Identification of fungal isolates that have inhibitory power >50% and have an inhibitory mechanism against pathogens

Isolate	Conidiophores	Metula	Phialid	Conidia	Genus
B2T1	Upright, branched, and hyaline	-	Shaped like a bottle	Round, chain-like, and hyaline	*Purpureocillium* sp.

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B3T7	Upright, branched, and thick	-	Shaped like a bottle	Round in clusters that form like flowers	*Trichoderma* sp.

Table 4. Continued

Isolate	Conidiophores	Metula	Phialid	Conidia	Genus
B1T2	Upright and branched	Upright and elongated	Shaped like a bottle	Round and chain-like	*Penicillium* sp.

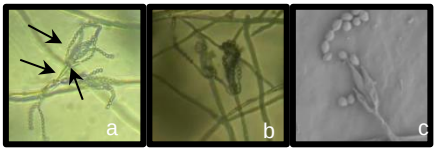


Figure 6. a. Microscopic identification of B2T1 isolate (Personal Documentation, 2024), b. (Ilmi, 2024), c. (Chen et al., 2024).

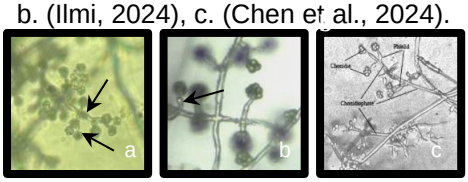
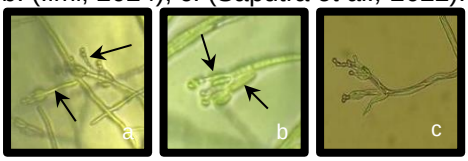


Figure 7. a. Microscopic identification of B3T7 isolate (Personal Documentation 2024), b. (Ilmi, 2024), c. (Saputra *et al.,* 2022).



Gambar 18. a and b Microscopic identification of B1T2 isolate (Personal Documentation, 2024), c. (Ilmi, 2024)

After antagonistic testing was carried out, isolate B2T1 has characteristics in the form of hyaline and branched conidiophores, no metula, bottle-shaped phialides, and round conidia and forms chains. Meanwhile, the results of macroscopic observations can be seen in (Table 1) where this isolate has characteristics in the form of light purple to grayish color with a round shape, convex surface and wavy edges. The results of microscopic and macroscopic observations refer to the genus Purpureocillium. According to Ahmad (2013) One species, *Purpureocillium lilacinum*, has the potential to inhibit pathogenic nematodes such as *Meloidogyne* spp. in plants. The characteristics of isolate B3T7 (Table 1) are green and white with a round circular shape, flat surface, and flat edges. The microscopic characteristics have thick and branched conidiophores, bottle-shaped phialides, and conidia clustered like flowers. This isolate belongs to the genus of Trichoderma fungi. According to Dalimunthe et al., (2019) in general, *Trichoderma* sp. fungi. has a symbiotic relationship with rubber plants. Amaria et al., (2013) argue that Trichoderma has a role as a biopesticide or biofertilizer because it releases secondary metabolites to suppress pathogen growth. This fungus has antimicrobial activity because it can produce β-glucanase and chitinase which are important hydrolytic enzymes in degrading pathogen cell walls. Isolate B1T2 macroscopically (Table 1) is yellowish white to brown and when the petri dish is turned over it is brown, the surface is irregularly round, convex and the edges are wavy. Microscopically this isolate has upright and branched conidiophores, long metula, bottle-like phialids, and round conidia and forms chains. The results of macroscopic and microscopic observations show that the isolate is a fungus from the genus Penicillium. According to Rozali (2015), *Penicillium* sp. fungi can increase plant growth and protect them from pathogen attacks through the antimicrobials they produce.

5. CONCLUSION

This study concludes that there are 15 pure fungal isolates which are then subjected to pathogenicity tests for the screening stage. Based on the test results, 7 isolates were obtained that were pathogenic and 8 non-pathogenic isolates. Furthermore, an inhibition test was carried out against the *Pestalotiopsis* sp. pathogen to determine the antagonism of the fungus. The results showed that there were 2 fungal isolates with codes B3T7 and B2T1 which were able to suppress the growth of pathogens with an inhibition percentage of 66.01% and 51.78%. In addition, isolate B1T2 showed an antibiosis interaction mechanism which can also be categorized as an antagonist. Further identification showed that the code B2T1 was the fungus *Purpureocillium* sp., isolate B3T7 was *Trichoderma* sp. while isolate B1T2 was *Penicillium* sp.

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