Identification of Pathogens Causing Leaf Blight on Teak in The Nursery PT Solusi Bangun Indonesia

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Abstract. Teak is commonly used as a revegetation plant because of its ability to live on critical land and its vigorous defense system against disease attacks. However, it must be recognized that teak plants can still be attacked by disease. This study aims to identify pathogens that cause leaf blight in teak in the nursery. This research method includes field observations using the census method and Koch's postulate test. Field observations showed that the area of leaf blight attack reached 93%, with widespread disease characteristics in brown necrotic areas. Koch's postulate found S1, S2, S3, and S4 isolates. The microscopic identification of pathogens showed that isolates S1 and S2 were *Rhizoctonia* sp. with characteristic characteristics of leafy hyphae adjacent to branching, a branching angle of 90°, no spores were found, and an irregular monyloid structure. Isolates S3 and S4 are *Curvularia* sp. with characteristics of hyphae that are septate. Conidia consists of 4-5 cells with one enlarged cell in the middle, and the cell color on the outside or tip tends to be transparent.

1 Introduction

Tectona grandis Linnaeus, commonly known as teak, from the family Verbenaceae, is used as a revegetation plant at PT. SBI. Superior teak varieties have relatively fast growth rates and high disease resistance, but field reports still indicate disease occurrences [1]. Teak in the Nursery stage of PT. SBI is susceptible to disease attack, resulting in decreased yield quality and quantity. The obstacle faced in teak propagation at PT SBI at the Nursery stage is a leaf blight attack. In research [2], teak plants in India were attacked by diseases at the nursery stage, with the highest attack intensity found in root rot and leaf blight. The intensity of leaf blight attacks on African wood seedlings at the BPDAS Citarum Ciliwung nursery reached 99.94%, with high attack intensity due to the young age of the seedlings, making them susceptible to disease [3]. Both biotic and abiotic factors can cause leaf blight symptoms. Fungi are a common biotic cause of leaf blight in nurseries [4]. Teak seedlings are vulnerable to pathogen attacks because they do not fully develop defense mechanisms against pathogens. According to, name [5], leaf pathogen infection can disrupt physiological (photosynthetic rate, stomatal conductance to CO2, water use efficiency, and leaf transpiration rate).

The severity of disease attacks significantly impacts the quality and quantity of plant production, especially in nurseries. Low-intensity disease attacks may have a small impact initially. Still, the inoculum can accumulate and cause disease outbreaks under favorable host conditions if left unchecked. Empowerment and protection are crucial to prevent and control pests and diseases. Pest and disease control in SBI nurseries has not been carried out, resulting in decreased productivity. The persistent occurrence of leaf blight in these nurseries has resulted in considerable economic losses, emphasizing the need for a comprehensive understanding of the causal agents. Protection against pests and diseases is essential due to the long life cycle of forestry plants [6]. Despite various management practices, the need for precision pathogen identification has hindered effective disease control measures [7]. This study aims to identify the fungus pathogens responsible for causing leaf blight in teak nurseries at PT. SBI.

2 Method

2.1 Time and location

The research was conducted from October 2023 to February 2024. Field data collection took place at the PT. SBI Nursery, Narogong Factory, Klapanunggal Sub-district, Bogor Regency, West Java. Pathogen identification was carried out in the Forest Pathology Laboratory, Faculty of Forestry and Environment, IPB.

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2.2 Procedure

2.2.1 Area of attack

The extent of the attack was assessed to measure the symptoms of leaf blight disease in teak using a census method on 328 teak plants at the PT. SBI Nursery. The extent of the attack was calculated using the formula [8]:

$$\text{The extent of infestation (\%)} = \frac{Number\ of\ plants\ affected}{\text{total number of plants observed}} \times 100\% \quad (1)$$

2.2.2 Sample collection

Samples were taken in the form of leaves showing symptoms of leaf blight. Leaf samples were randomly collected at the PT. SBI Nursery. The leaves were transported to the Forest Pathology Laboratory for further research. Transport was done by placing the leaves in ziplock bags with silica gel. Silica gel was used as an alternative storage and transport method to maintain humidity [9]. Isolation Sample Plant Pain and Purification Isolation was done by cutting between the infected and uninfected tissues, with a size of ± 0.5 cm. The leaf pieces were sterilized in a beaker glass containing 70% alcohol for 2 minutes to eliminate surface contamination, and then the samples were in sterile water three times. The samples were then dried using sterile filter papers. The sterilized leaf pieces were placed on PDA media and incubated for ± 7 days, followed by the purification and proliferation of isolates. The fungal isolates were purified by taking a small amount of fungal mycelium grown on PDA media [10]. This procedure was performed in an LAF to maintain sterility. Isolate growth was measured by the radial growth of the fungal diameter Figure 1. Growth was calculated using the formula :

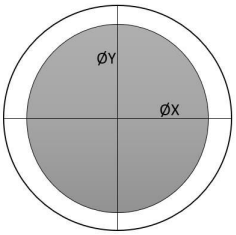

Fig. 1. Observation of isolates' growth diameter [11]

$$\text{Diameter radial direction} = \frac{ØX.ØY}{2} \times 100\% \quad (2)$$

Information :
ØX = diameter of X-axis (cm)
ØY = diameter of Y-axis (cm)

2.2.3 Inoculation

Inoculation aimed to prove that the tested isolate was the causal agent of the observed disease symptoms. The isolation results were inoculated into the host organism to test pathogenicity [12]. This test aimed to confirm that the isolate was a pathogen causing leaf blight in its host plant. The test plants were ± 6-month-old teak seedlings. Inoculation was performed by applying carborundum powder and placing the isolate. The symptomatic parts of the plant were re-isolated on PDA media and compared with the initial isolation results.

2.2.4 Identification of Pathogens

The fungi were identified macroscopically and microscopically. Riddle's preparation was performed to observe the microscopic structure of the isolates comprehensively. Microscopic observations included the presence of septa in the hyphae, hyphal growth characteristics, hyphal branching, conidia, and conidiophore morphology. The identification process used a light microscope with a 40x magnification.

2.3 Data analysis

Field and laboratory research data obtained were then analyzed descriptively. The data were then compared with existing literature.

3 Results

Based on census results, the comprehensive attack symptom of teak leaf blight reaches 93%. The census results can be seen in Table 1.

Table 1. Census results teak leaf blight Nursery PT. SBI

Description	Numbers
Healthy	21
Diseased	307
Total	328

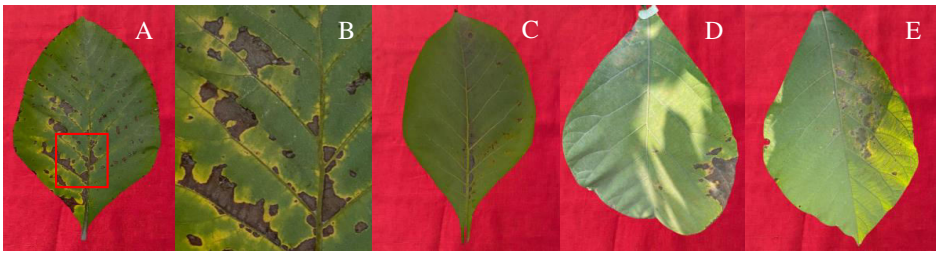

Fig. 2. Teak Leaves Affected by Leaf Blight: Sample S1 (A), Insert of Leaf Blight Symptoms on S1 (B), Sample S2 (C), Sample S3 (D), Sample S4

Leaf blight symptoms can be seen in Figure 2, characterized by areas of necrotic leaves that are dry and brown with yellow edges, which tend to spread. Observations indicated that the progression from leaf spot to leaf blight is marked by an expansion of necrotic brown areas, yellowing at the edges, and spreading black spots, ultimately causing the leaves to dry, curl, and fall off. These symptoms are consistent with those who described rapidly spreading brown spots leading to leaf desiccation and drop. The leaf drop is attributed to necrosis caused by leaf blight, which spreads and results in the death of the entire leaf organ.

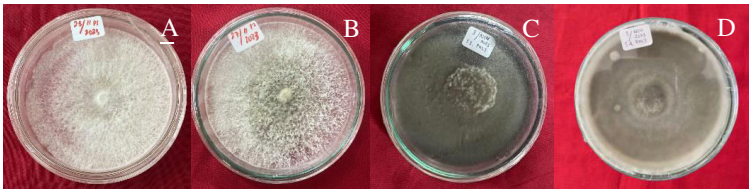

Fig. 3. Isolation Results: (A) isolate S1, (B) isolate S2, (C) isolate S3, (D) isolate S4

The isolation of leaf blight disease symptoms resulted in four pure culture samples suspected to be the pathogens causing teak leaf blight. The isolation results are shown in Figure 3. Isolates S1 and S2 showed white colonies on the upper surface and white to yellowish colonies on the lower surface, with a mountainous surface texture. Concentric rings were visible from the center of the colony towards the edge. Isolate S1 has hyphae spreading in a wavy pattern, thickening in the central area and thinning at the edges. Isolate S2 has hyphae spreading in a circular pattern, thickening in the center and thinning at the edges. Initially, isolates S1 and S2 were white but turned yellowish-brown over time. Isolate S3 has yellowish-brown colonies on both the upper and lower surfaces, which turn black as they age. It exhibited concentric rings, thickening at the colony center resembling a mound, thinning at the edges, and forming concentric circles. Isolate S4 showed grey colonies on the upper surface and black on the lower surface, which darkened and turned black with age. Concentric rings were visible from the colony center to the edges. During each isolate's growth, the fungal colonies' color change was observed to occur with increasing age. Initially white, the hyphae change color over time.

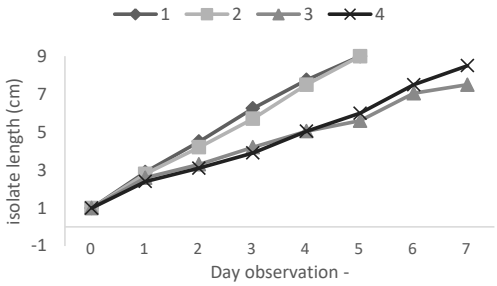

Fig 4. Diameter Growth Graph of Isolates

. The growth graph of isolates S1 and S2 showed faster growth than isolates S3 and S4, as shown in Figure 4. Observations indicated that isolates S3 and S4 took longer to fill the petri dish due to vertical growth during the growth period, resulting in slower growth. The growth in diameter of a fungus is influenced by its ability to undergo the reproductive phase.

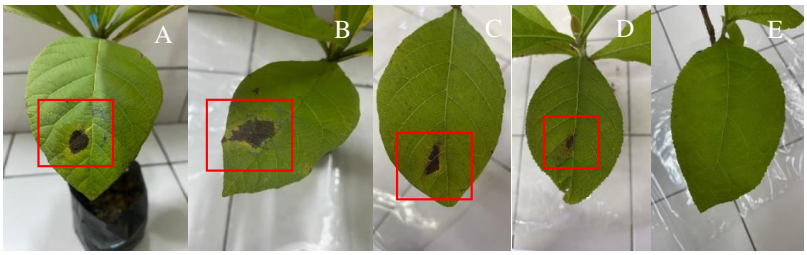

Fig. 5. Inoculation results on day 7 (A) isolate S1, (B) isolate S2, (C) isolate S3, (D) isolate S4, (E) without

Inoculation is a series of steps used to test whether a fungus can cause disease. Inoculation results on teak plants showed symptoms similar to those on the plants from which the isolates were taken. Symptoms of leaf blight appeared on leaf samples given the isolate, which proves that the fungus can cause disease.

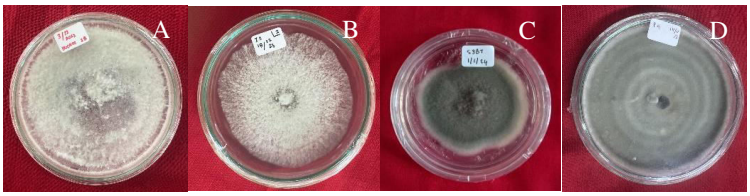

Fig. 6. Re-isolation Results of Isolate S1 (A), Isolate S2 (B), Isolate S3 (C), Isolate S4 (D)

Based on Figure 6, the re-isolation results show that the fungus exhibited the same macroscopic characteristics as the initial isolate samples. No fungal growth was observed in the control re-isolation media. This confirms that biotic factors were responsible for the symptoms.

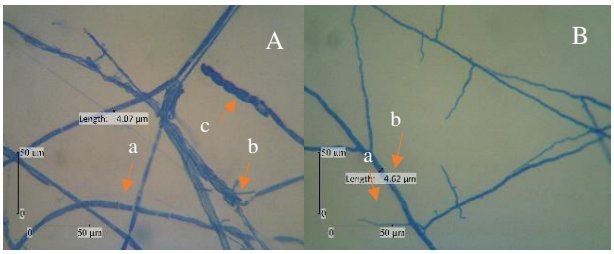

Fig. 7. Microscopic Observation of Isolate S1 (A) and S2 (B). Red arrows indicate septate hyphae (a), 90° branching (b), and monoid cells (c)

Microscopic observations indicated that samples S1 and S2 were characterized by septate hyphae and septa near the branches. The samples exhibited 90° branching angles and an absence of spores. Monilioid structures were present, indicating an early stage of sclerotium formation.

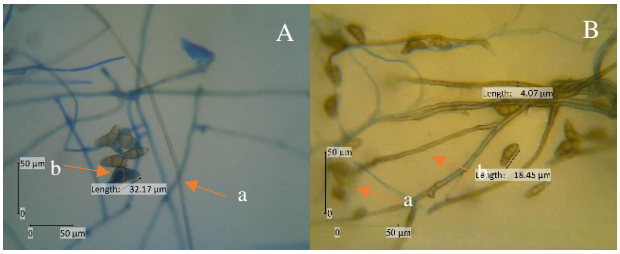

Fig. 8. Microscopic Observation of Isolate S3 (A) and Isolate S4 (B). Red arrows indicate septate hyphae (a) and conidia (b)

Isolates S3 and S4 were characterized by septate hyphae and septate conidia consisting of 4-5 cells, with obovoid (curved), atypical (straight with tapered ends), oval elongated, and curved shapes, with one cell enlarged in the middle. Cell color on the outside or tip tends to be transparent

4 Discussion

The high extent of the attack is attributed to the compatibility of the host with the environmental conditions, the vulnerability of leaf tissues at the nursery stage, and the virulence of the pathogens. According to [10], disease symptoms can develop relatively quickly in the upper parts of the plant compared to the base due to the more complex tissue structure of the stem compared to the tips. This finding aligns with [13], who reported that the disease can spread more rapidly when a pathogen finds a susceptible host location. Generally, diseases can be caused by biotic and abiotic factors. Based on observations, leaf blight disease spread almost throughout the observation area, indicating that biotic factors caused the symptoms. Fungi are one of the causes of leaf blight in teak seedlings [14]. Fungi can cause necrosis in plant tissues, with necrotic leaves turning yellow to brown, indicating tissue death [15]. An increase or decrease in chlorophyll content can indicate the level of resistance to various plant diseases [16]. White threads on teak leaves were suspected to be hyphae of the leaf blight pathogen.

Inoculation is a series of steps to test whether a fungus can cause disease. Fungi exhibit highly effective hyphal penetration of host tissues, using extracellular enzymes to dissolve their substrates and transport simple products such as sugars or amino acids [17]. During the inoculation process, fungal hyphae were observed to penetrate the leaf tissues, developing typical blight symptoms effectively [18]. The inoculation stage was conducted using the attachment method with the four obtained isolates. The inoculation results are shown in Figure 5. The inoculation results on teak plants showed symptoms similar to those observed on the plants from which the isolates were taken. Leaf blight symptoms appeared on the leaf samples treated with the isolates, proving that the fungus could cause disease. During the observation period, the inoculation results for isolates S1 and S2 showed symptoms on the 3rd day, significant disease development, and leaves fall by the 11th day. Leaf fall was due to extensive necrosis and increased disease intensity. Isolates S3 and S4 begin showing symptoms on the sixth day, with leaves falling on the 21st, indicating slower disease progression. The differing pathogenicity characteristics can be attributed to the interaction between the pathogen's ability to attack the host. Differences in pathogenicity may be due to pathogen virulence, host resistance, pathogen development, and environmental conditions such as temperature and humidity [19].

Microscopic observations indicated that samples S1 and S2 were *Rhizoctonia* sp. This is consistent with [14], who described *Rhizoctonia* sp. as having perpendicular branching, septate hyphae, and monilioid structures without spores. Monilioid structures are chains of cells with varying shapes [20]. Microscopic observations are shown in Figure 7. Based on microscopic observations, *Rhizoctonia* sp. mycelium is classified as sterile. The sterile mycelium of *Rhizoctonia* sp. is a delicate thread-like structure produced by fungi without involving asexual reproductive structures, consistent with the absence of spores in microscopic observations [21]. This matches previous findings by [22], describing *Rhizoctonia* sp. mycelium as sterile. Sclerotium is a moniloid cell collection that grows spread out with irregular shapes and sizes. It is black, and there is air dew with fine white hairs. This is according to the research of [23], which shows that the sclerotium of *Rhizoctonia* sp. is irregular and blackish-dark in color. States that *Rhizoctonia* sp. Isolates S3 and S4 were identified as *Curvularia* sp. The outer cells or ends tend to be transparent, as shown in Figure 9. This matches the study by [24], which described *Curvularia* sp. as having septate hyphae and conidia consisting of 3-5 cells, typically curved and darker in the middle. *Curvularia* sp. produces spores during growth, aiding disease spread through the air or nearby planting areas. Macroscopically, colony color differed on the upper and lower surfaces, but microscopically, the hyphae and conidia shapes were similar. Macroscopic differences are due to the different species of *Curvularia* sp.

5 Conclusion

The incidence of leaf blight disease at the PT. SBI Nursery was found to be 93%. This high percentage was facilitated by the favorable environmental and host conditions that promoted fungal spread. Based on macroscopic and microscopic characteristics, fungal identification to the genus level indicated that isolates S1 and S2 were *Rhizoctonia* sp., while isolates S3 and S4 were *Curvularia* sp.

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