

## RESEARCH ARTICLE



## Evaluation of Leaf Spot and Blight Diseases in Mahogany (*Swietenia mahagoni*) Seedlings in Rumpin Nursery, Bogor Regency, Indonesia

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### ABSTRACT

Leaf spot and blight diseases are major factors limiting forest seedling production in nurseries, including economically important species, such as mahogany (*Swietenia mahagoni*). However, specific information on their incidence, severity, and causative pathogens remains limited. This study aimed to evaluate the incidence and severity of the diseases and identify the pathogenic fungi associated with mahogany seedlings at Rumpin Nursery, Bogor Regency. Observations were conducted on 3- and 4-month-old seedlings in the open growth area, with a 10% sampling intensity. Leaf samples showing symptoms were isolated, tested using Koch's postulate, and analyzed using ANOVA. Pathogen identification was performed through both morphological characterization and molecular analysis using ITS rDNA sequencing. The results showed a very high incidence of disease, namely 95.44% (3 months old) and 92.66% (4 months old), with a moderately severe severity (44.72% and 44.20%). Five types of pathogenic fungi were identified, namely *Corynespora cassiicola*, *Pseudopectinotopsis theae*, *Colletotrichum fruticola*, *Colletotrichum gloeosporioides*, and *Bipolaris setariae*. The presence of pathogens with a wide host range, the ability to survive saprophytically, and their capacity to spread through water and air suggest that nurseries can serve as sources of inoculum and pathways for pathogen dissemination. Therefore, integrated disease management should be emphasized, incorporating sanitation, optimal spacing, irrigation control, and the use of biological agents.

### Introduction

Mahogany (*Swietenia mahagoni*) is one of the most valuable timber species in both domestic and international markets [1]. Its wood is widely recognized as a raw material for furniture, construction, and crafts due to its good mechanical properties, fine-grained texture, and beautiful color. It has been cultivated in Indonesia, especially on the island of Java, since the Dutch colonial era [2–4]. Mahogany wood is classified as durability class III and strength class II-III, and included in the beautiful wood group, as per the Decree of the Minister of Forestry No. 163/Kpts-II/2003 [5]. In addition to the wood, various parts of the mahogany plant also have uses, including the fruit as a traditional medicine and natural pesticide, the fruit skin as a natural dye, the sap as an adhesive, and the leaves as animal feed [6,7]. The high economic value and diverse uses of mahogany require the availability of healthy and high-quality seedlings as the basis for its sustainable development.

The availability of healthy and high-quality mahogany seedlings remains a significant challenge at the nursery stage. Mahogany seedlings are susceptible to leaf spot and blight diseases. These diseases cause necrotic spots on leaves, rapid symptom development, physiological dysfunction, and partial or total tissue death in susceptible seedlings [8]. If not effectively controlled, these diseases can reduce the rate of photosynthesis, inhibit growth, decrease seedling quality, and in severe cases, cause seedling death in nurseries [9]. However,

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monitoring and evaluation of leaf diseases in nursery seedlings have been widely conducted; scientific information on the identification of pathogens causing leaf spot and blight diseases, especially in mahogany seedlings, remains very limited. Therefore, an evaluation of leaf spot and blight diseases in mahogany seedlings in nurseries is needed, and the identification of the causative pathogens must be carried out so that appropriate disease control measures can be promptly determined.

## Materials and Methods

### Research Location and Time

The research was conducted from January to April 2025. Data collection on disease incidence and severity in seedlings was carried out at Open Growth Area 3, Rumpin Nursery, Bogor Regency. Koch's postulate testing, pathogen identification, and data analysis were carried out at the Forest Pathology Laboratory, IPB University.

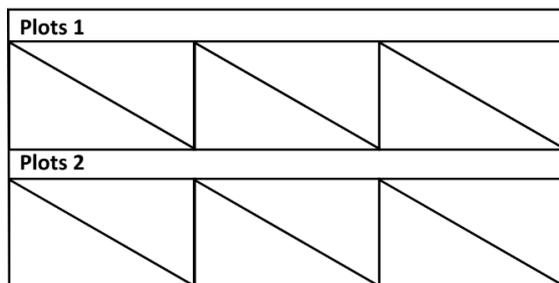
### Tools and Materials

The tools used in this study included a microscope, a laminar air flow, a PCR machine, and a laptop with SAS software version 21.0.16, BioEdit 5.0.9, and MEGA 12.0.15. The materials used included leaf tissue affected by leaf spot and blight diseases, potato dextrose agar (PDA) medium, Gsyne DNA Extraction Kit, ITA1 and ITS4 primers, and mahogany seedlings aged 3 and 4 months, with an average height of 20–35 cm and a diameter of 0.3–0.7 cm. The seedlings were planted in polybags containing cocopeat (80%) and husk (20%) media with rock phosphate and NPK SK cote fertilizer and watered automatically twice a day.

### Research Procedure

#### Evaluation of Leaf Spot and Blight Diseases

The evaluation began with observing climate elements in the Rumpin Nursery area. The parameters observed were rainfall, temperature, and humidity, which were accessed from the official website of the Meteorology, Climatology, and Geophysics Agency (*Badan Meteorologi, Klimatologi, dan Geofisika/BMKG*). Observations of leaf spots and blight were carried out directly by observing the shape, size, and color of the symptoms found on mahogany leaves. The layout of the observation plots and sampling design is presented in Figure 1.



**Figure 1.** Observation plot for leaf spot and blight diseases on mahogany seedlings (*Swietenia mahagoni*) at the Rumpin Nursery, Bogor Regency. The intensity and severity of the disease were observed in two sample plots (3- and 4-month-old seedlings) that were repeated three times. Each plot had a population of 1680 seedlings. Observations were made along the longest diagonal line with a sampling intensity 10%.

#### Evaluation of Disease Incidence and Severity

Disease incidence is calculated as the ratio of the number of seedlings affected by disease to the total number of seedlings observed. Disease severity is calculated based on the number of seedlings at each damage score multiplied by the score value, divided by the product of the total number of seedlings observed and the highest damage score. Leaf damage scores are set on a scale of 1–5 [10]. Disease incidence and severity are calculated using Equations 1 and 2 [11].

$$\text{Disease Incidence} = \frac{\text{Number of infected seedlings}}{\text{Total seedlings observed}} \times 100\% \quad (1)$$

$$\text{DS} = \frac{\sum(n \times v)}{N \times V} \times 100\% \quad (2)$$

Information:

- DS : percentage of disease severity  
n : number of seedlings affected at the i-th disease score,  
v : symptom score at the i-th classification,  
N : total number of seedlings observed, and  
V : highest score value used in the scoring classification.

### **Isolation and Koch's Postulates Test**

Isolation was performed by surface-sterilizing leaf sections (5–10 mm<sup>2</sup>) using 70% alcohol three times, followed by rinsing with sterile water. Leaf tissue was placed on PDA medium and incubated at room temperature for 7 days. Different colonies were transferred to new petri dishes to obtain pure cultures [8]. Koch's postulates were tested by inoculating each isolated pathogen onto healthy 3-month-old mahogany seedlings. The seedlings were wounded using carborundum powder, and subsequently, the incubation period and disease symptom development.

### **Pathogen Identification**

Pathogen identification employed morphological and molecular approaches. Morphological identification relied on macroscopic characteristics (colony color, texture, shape, and size) [12] and microscopic characteristics (spores, hyphae, and other reproductive structures), with comparisons made using standard fungal identification keys, such as those described by Watanabe [13]. Molecular identification is performed through analysis of the Internal Transcribed Spacer (ITS) region sequence using the Gsyne DNA Extraction Kit (GenAid) with universal primers ITS1 and ITS4. The PCR results are sent to 1<sup>st</sup> base (Malaysia) through PT. Genetika Science Indonesia for sequencing. Sequence data were edited and analyzed for quality using BioEdit software version 5.0.9, then compared with the NCBI database using the BLAST program. The phylogenetic relationships among isolates were analyzed by constructing a phylogenetic tree using Mega software version 12 with the maximum likelihood method [14].

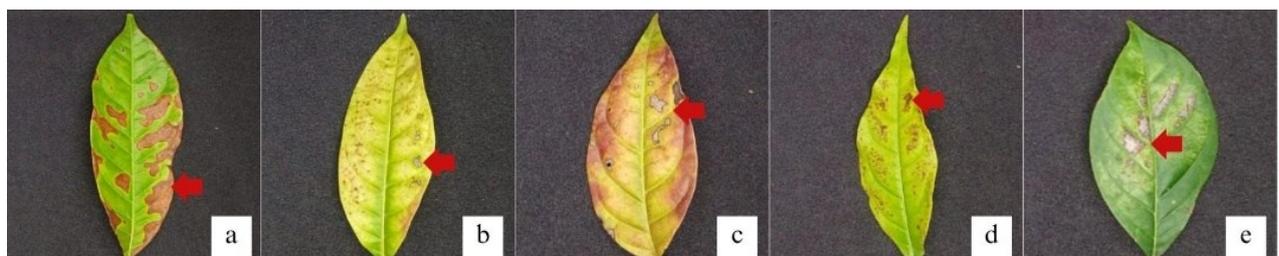
### **Data Analysis**

Incidence and severity data for leaf spot and blight diseases in 3- and 4-month-old mahogany seedlings were analyzed using analysis of variance (ANOVA) at a significance level of 5%. Data were analyzed using three replicates for each seedling age group. When significant effects were detected, Duncan's Multiple Range Test (DMRT) was applied.

## **Results**

### **Symptoms of Leaf Spots and Blight on Mahogany Seedlings**

Based on observations of disease symptoms in mahogany seedlings, leaf samples showing symptoms of leaf spots and leaf blight were collected. Sampling was conducted using purposive sampling to represent the variety of symptoms that appeared in all observation plots at the research site. A total of five samples were selected, consisting of two samples with leaf spot symptoms and three samples with leaf blight symptoms (Figure 2).



**Figure 2.** Samples of mahogany seedlings with leaf spot and blight symptoms at the Rumpin Nursery. Leaf blight symptoms (a, c, and e) appear as spreading necrosis, brown in colour, irregular in shape, with dried leaf tissue, and a relatively rapid rate of symptom development. In contrast, leaf spot symptoms (b and d) are characterized by light to dark brown spots, irregularly round in shape, with a slower rate of symptom development.

### Incidence and Severity of Disease

The incidence of leaf spot and blight diseases in 3-month-old mahogany seedlings reached 95.44%, higher than that in 4-month-old seedlings at 92.66%. The severity of the disease in 3-month-old seedlings was 44.72%, slightly higher than that in 4-month-old seedlings at 44.20%. The severity of disease in both age groups was classified as moderately severe (Table 1). Analysis of variance showed that seedling age didn't have a significant effect on the incidence or severity of leaf spot and blight ( $p > 0.05$ ) (Table 2).

**Table 1.** Effect of seedling age on the incidence and severity of leaf spot and blight disease in mahogany seedlings at Rumpin Nursery. Values are expressed as percentages based on visual assessment of disease symptoms. Assessments were conducted on seedlings aged 3 and 4 months.

Age	Incidence of disease	Severity of disease
3 months	95.44%	44.72%
4 months	92.66%	44.20%

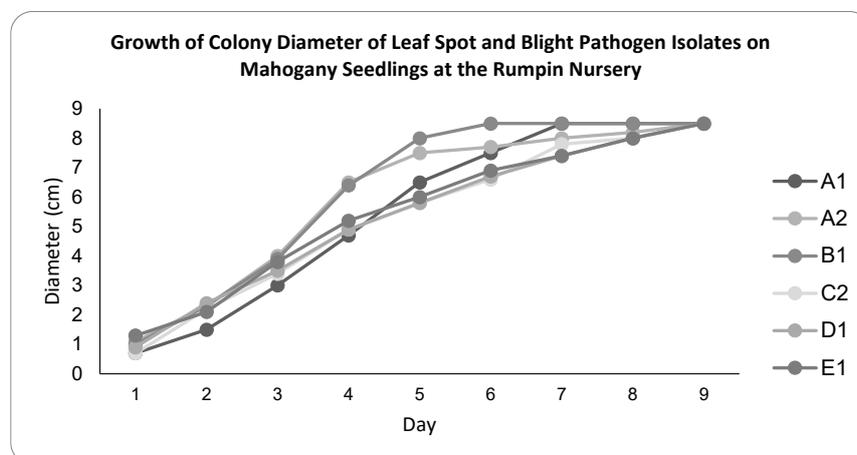
**Table 2.** Results of analysis of variance (ANOVA) on the incidence and severity of disease in mahogany seedlings. Data from 3- and 4-month-old mahogany seedlings at the Rumpin Nursery were analyzed. ANOVA was used to evaluate the effect of seedling age on disease incidence and severity.

No	Variable	<i>P-value</i>
1	Incidence of disease	
	Seedlings age 3 months	0.5038 <sup>u</sup>
	Seedlings age 4 months	0.5038 <sup>u</sup>
2	Disease severity	
	Seedlings age 3 months	0.9093 <sup>u</sup>
	Seedlings age 4 months	0.0993 <sup>u</sup>

\*<sup>u</sup> treatment had no real effect on the 5% test level

### Isolation and Koch's Postulate Test

The fungal isolation results produced six isolates with different characteristics, including color, texture, and colony growth patterns. The diameter of the pathogenic isolate colonies was measured daily until the colonies filled the petri dishes (Figure 3). Each isolate was inoculated onto healthy mahogany seedlings to evaluate pathogenicity and disease incubation period (Figure 4).



**Figure 3.** Growth rate of colony diameter of pathogen isolates causing leaf spot and blight diseases in mahogany seedlings. The growth of colony diameter of isolates increased rapidly on days 1 to 5 of the incubation period. All isolates had filled petri dishes with a diameter of 8.5 cm on the 9<sup>th</sup> day of observation.



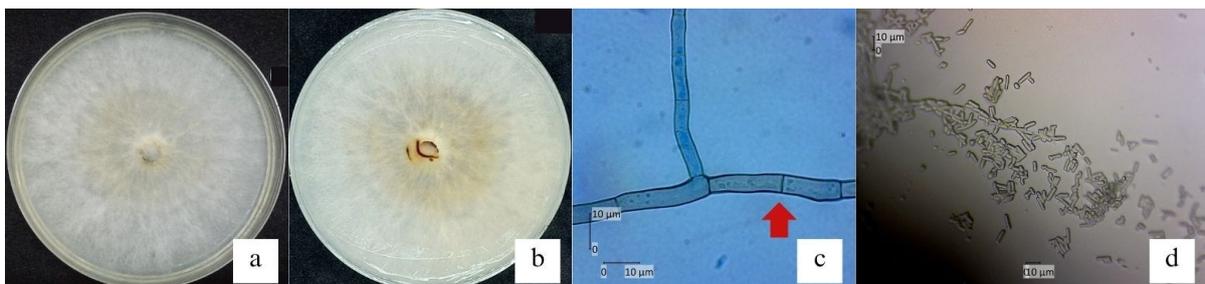
**Figure 4.** Results of inoculation of each pathogen isolate on healthy 3-month-old mahogany seedlings. All isolates tested showed pathogenic properties against healthy mahogany seedlings. The inoculated pathogens had an average incubation period of 1-2 days after inoculation. This short incubation period indicates the pathogen’s ability to infect hosts quickly under favorable environmental conditions.

### Pathogen Identification

Morphological identification indicated that all pathogen isolates possessed septate hyphae and produced conidia as reproductive structures. Molecular identification using DNA sequence analysis and phylogenetic tree construction confirmed the morphological identification results. Based on the results of morphological and molecular identification, five types of pathogens were identified, namely *Corynespora cassiicola* (isolate A1) (Figure 5 and 6), *Pseudopezalotiopsis theae* (isolates A2 and B1) (Figure 7 and 8), *Colletotrichum fruticola* (isolate C1) (Figure 9 and 10), *Colletotrichum gleosporioides* (isolate D1) (Figure 9 and 11), and *Bipolaris setariae* (isolate E1) (Figure 12 and 13). These results demonstrate that leaf spot and blight diseases in the Rumpin Nursery are caused by multiple pathogens, with five types showing relatively short incubation periods of 1–2 days, allowing the disease to develop rapidly.

Seedling conditions and the nursery environment also influence the development of the disease. Young mahogany seedlings, planted in large numbers with very close spacing and even intertwined root systems, facilitate pathogen infection and spread among plants. In addition, environmental conditions at the Rumpin Nursery are conducive to pathogen development, as indicated by an average annual rainfall of 3,032.45 mm, daily temperatures ranging from 22 to 30 °C, and relative humidity levels of 72-98%. High rainfall, accompanied by wind, human and insect activity, further facilitates the spread of inoculum within the nursery area.

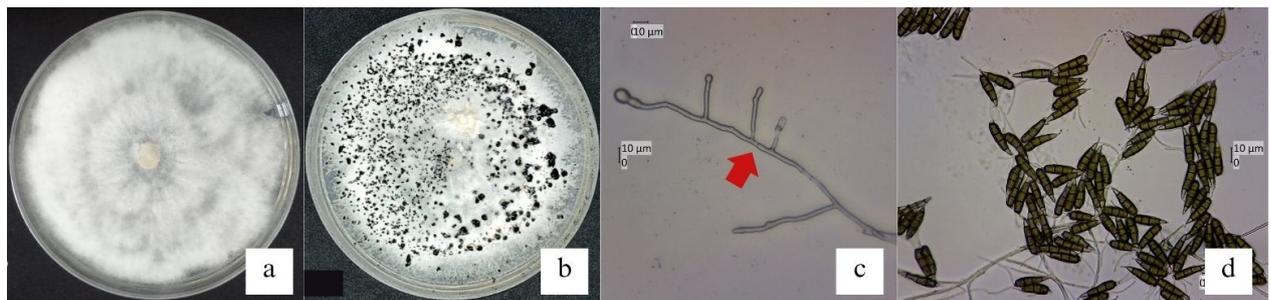
In addition to environmental factors, differences in the biological characteristics of pathogens also play a role in disease dynamics. Variations in conidial size, shape, and cell number influence the pathogen’s ability to penetrate plant tissues, develop internally, and determine its lifestyle, including parasitic or saprophytic and obligate or facultative modes. The growth pattern of the colony’s mycelium, which tends to spread sideways, indicates the pathogen’s potential to spread widely to other plants rather than intensively attacking a single seedling. These differences in characteristics suggest that plant responses and disease control strategies should be tailored to the specific characteristics of each pathogen involved.



**Figure 5.** Morphological description of isolate A1 (*Corynespora cassiicola*). Macroscopically (a–b), the colony of isolate A1 is white with a yellowish center, has a smooth texture resembling cotton, and shows radial growth toward the edge of the petri dish. Microscopically (c–d), the isolate exhibited septate hyphae and cylindrical conidia measuring 7.5–15 µm in length and 2.5–7.5 µm in width.



**Figure 6.** Phylogenetic tree of *Corynespora cassiicola* based on ITS nucleotide sequences constructed using the Maximum Likelihood method. Isolate A1 clusters with the reference sequence of *C. cassiicola* from GenBank. This grouping indicates that isolate A1 is identified as *Corynespora cassiicola* and is consistent with the observed morphological characteristics.



**Figure 7.** Identification results of isolates A2 and B1 (*Pseudopestalotiopsis theae*). Macroscopically (a–b), isolates A2 and B1 are white, smooth in texture, and show growth resembling blooming flowers. Black conidiomata are scattered irregularly on older isolates. Microscopically (c–d), these isolates exhibited septate hyphae and fusiform conidia composed of five cells, with an average length of 26.82 µm and a width of 6.17 µm.



**Figure 8.** Phylogenetic tree of *Pseudopestalotiopsis theae* based on ITS nucleotide sequences constructed using the Maximum Likelihood method. Isolate A2 and B1 clusters with the reference sequence of *P. theae* from GenBank. This grouping indicates that isolates A2 and B1 are identified as *Pseudopestalotiopsis theae* and are consistent with the observed morphological characteristics.



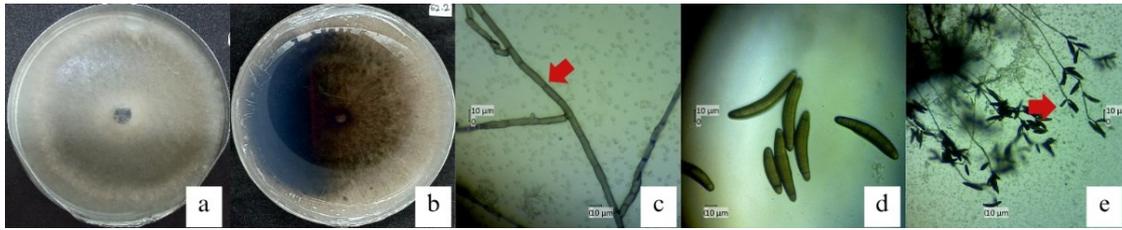
**Figure 9.** Identification results of isolate C1 and D1 (*Colletotrichum fruticola* and *Colletotrichum gleosporioides*). Macroscopically (a–b; e–f), the colonies of isolates C1 and D1 are grayish-black in color, smooth in texture, and show radial growth toward the edge of the petri dish. Microscopically (c–d; g–h), these isolates have septate hyphae with cylindrical conidia measuring 10–16 µm in length and 5–7 µm in width.



**Figure 10.** Phylogenetic tree of *Colletotrichum fruticola* based on ITS nucleotide sequences constructed using the Maximum Likelihood method. Isolate C1 clusters with the reference sequence of *C. fruticola* from GenBank. This grouping indicates that isolate C1 is identified as *Colletotrichum fruticola* and is consistent with the observed morphological characteristics.



**Figure 11.** Phylogenetic tree of *Colletotrichum gleosporioides* based on ITS nucleotide sequences constructed using the Maximum Likelihood method. Isolate D1 clusters with the reference sequence of *C. gleosporioides* from GenBank. This grouping indicates that isolate D1 is identified as *Colletotrichum gleosporioides* and is consistent with the observed morphological characteristics.



**Figure 12.** Identification results of isolate E1 (*Bipolaris setariae*). Macroscopically (a–b), the colony of isolate E1 is grayish brown in color, smooth in texture, and shows radial growth towards the edge of the petri dish. Microscopically (c–d), this isolate exhibited septate hyphae with cylindrical conidia consisting of 8 cells with an average length of 59.38 µm and a width of 16.37 µm. *Bipolaris setariae* has brown, erect conidiophores with conidia at their tips.



**Figure 13.** Phylogenetic tree of *Bipolaris setariae* based on ITS nucleotide sequences constructed using the Maximum Likelihood method. Isolate E1 clusters with the reference sequence of *B. setariae* from GenBank. This grouping indicates that isolate E1 is identified as *Bipolaris setariae* and is consistent with the observed morphological characteristics.

## Discussion

### Interaction between Host, Pathogen, and Environment in the Development of Leaf Spot and Blight Diseases in Mahogany Seedlings

The high incidence rate of disease in 3- and 4-month-old mahogany seedlings (92.66–95.44%) indicates that the spread of disease is widespread in nurseries. This condition is thought to be influenced by the close spacing between seedlings, which increases the chance of contact between infected plant tissue and healthy plants, thereby accelerating disease transmission [15]. Young seedlings are more susceptible to infection because they are at early growth stages, with tissue structures and cell walls that are not fully developed [11]. Disease spread and development are influenced by interactions among host, pathogen, and environmental factors. Plant disease develops when a susceptible host, a virulent pathogen, and favorable environmental conditions are present. The Rumpin Nursery environment is characterized by high rainfall (3,032.45 mm per year), temperatures ranging from 22–30 °C, and high relative humidity (72–98%), which are optimal conditions for the development of pathogenic fungi [8]. Therefore, the high incidence of disease in mahogany seedlings at the study site is likely the result of interactions among the susceptibility of young seedlings, close seedling spacing, and environmental conditions conducive to pathogen growth.

The percentage of disease severity in 3- and 4-month-old mahogany seedlings reached 44% and was classified as moderately severe. This percentage was lower than the disease incidence. This condition is thought to occur due to the effective maintenance practices at the Rumpin Nursery, particularly the implementation of automatic watering irrigation with water volumes adjusted to seedling requirements and an effective polybag drainage system, thereby preventing water stagnation and maintaining appropriate environmental humidity. This management can limit the duration of leaf wetness, a key factor in symptom development and pathogen sporulation. According to Febbiyanti [16], prolonged leaf wetness, high humidity, as well as rainfall and wind, play important roles in accelerating sporulation and pathogen spore dispersal. Therefore, effective maintenance practices contribute to reducing disease severity despite a high incidence of infection.

## Variations of Pathogens Found

Five identified pathogens cause leaf spot and blight diseases in mahogany seedlings, namely *Corynespora cassiicola*, *Pseudopestalotiopsis theae*, *Colletotrichum fruticola*, *Colletotrichum gloeosporioides*, and *Bipolaris setariae*. *Corynespora cassiicola* is the main pathogen that causes leaf disease. This pathogen is widespread in tropical and subtropical regions and has a very broad host range, covering more than 530 plant species from 400 different genera, including agricultural and forestry plants [17–20]. *Pseudopestalotiopsis theae* is a primary cause of leaf fall disease in rubber plants, which affected 22,000 ha of rubber plantations in 2018 and increased to 382,000 ha in 2019 [21,22], in Indonesia, Malaysia, Sri Lanka, India, and Thailand. *Colletotrichum fruticola* and *Colletotrichum gloeosporioides* are known to cause anthracnose disease in a wide range of hosts, including agricultural and forestry plants. Its distribution is cosmopolitan, especially in tropical and subtropical regions with high humidity [23–26].

*Bipolaris setariae* is a pathogen that commonly infects cereal crops, including rice, corn, and wheat. It has been reported in more than 60 plant genera [27–29]. This suggests that host shifting can occur when pathogens encounter similarities in physiological or biochemical conditions between cereal crops and woody plants, particularly in high-humidity environments or when plants are under stress. These findings suggest that nurseries can act as a conduit for the spread of pathogens between agricultural and forestry systems, thereby increasing the risk of pathogen transmission between plant species. These five types of pathogens belong to the class Ascomycota and can live saprophytically, meaning they can survive on dead organic matter or live without a host [30]. Pathogens infect plants through splashes-dispersed conidia and the airborne dispersal of ascospores [19,21,24].

## Practical implications and recommendations for disease management in nurseries

Integrated disease management is essential considering that *Corynespora cassiicola*, *Pseudopestalotiopsis theae*, *Colletotrichum fruticola*, *Colletotrichum gloeosporioides*, and *Bipolaris setariae* can survive saprophytically on plant debris and spread through water and air. Effective strategies include nursery sanitation, wider spacing of seedlings, controlled watering, and removal of infected plants to reduce humidity and eliminate inoculum sources. Biological agents such as *Trichoderma* sp. offer eco-friendly suppression of pathogens and should be integrated with minimal fungicide use to maintain nursery health [8,24,28]

## Limitations of the study

This study was limited to observing seedlings aged 3 and 4 months, so it cannot describe the vulnerability of seedlings at older ages. The relatively short observation period also did not allow for the evaluation of disease dynamics across different seasons. Therefore, further research involving a wider range of seedling ages and a longer observation period is highly recommended to better understand disease development, host-pathogen interaction, and environmental influences on disease progression.

## Conclusions

This study is the first evaluation of leaf spot and blight diseases in mahogany seedlings (*Swietenia mahagoni*) at the Rumpin Nursery. The results showed that the incidence of disease was very high in both seedling ages observed (95.44% at 3 months and 92.66% at 4 months), with a moderately severe level of severity (44.72% and 44.20%). Five pathogenic fungi were identified based on morphological and molecular (ITS rDNA sequencing) analyses, namely *Corynespora cassiicola*, *Pseudopestalotiopsis theae*, *Colletotrichum fruticola*, *Colletotrichum gloeosporioides*, and *Bipolaris setariae*. The presence of pathogens with a wide host range, their capacity to survive as saprophytes, and their dispersal through water and air indicate that nurseries can act as sources of inoculum and as bridges for pathogen spread between plant species and between forestry and agricultural systems. Therefore, the implementation of integrated disease management is essential, including improved nursery sanitation, increased seedling spacing, irrigation management, and the removal of infected seedlings. The use of biological agents, such as *Trichoderma* sp., combined with the limited use of fungicides, is recommended to support sustainable disease management and maintain the health of mahogany seedlings.

## Author Contributions

**NSI:** Data collection, analysis, writing – original draft; **YI:** Supervision, validation – final draft; **ASW:** Supervision; **NEL:** Supervision.

## AI Writing Statement

The author acknowledges the use of ChatGPT (OpenAI) for assistance in language editing and improving text clarity during manuscript preparation. The author has carefully reviewed and revised the content generated and takes full responsibility for the scientific accuracy and integrity of the final manuscript.

## Conflicts of interest

There are no conflicts to declare.

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