Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2026, Vol. 26 (No. 1), e0265486

Research article

Ceratocystis fimbriata Causing Wilt and Sudden Death on *Acacia mangium* in South Sumatera

Rahmat Pratama[1], Ahmad Muslim[1]*, Nurhayati Damiri[1], Harman Hamidson[1], Suwandi[1] and Rizki Putri Amelia [2]

[1]Phytopathology Laboratory, Plant Protection Study Program, Faculty of Agriculture, Universitas Sriwijaya. Jl. Raya Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia
[2]Department of Plant Science, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa 524 Ilir Barat I, South Sumatera, Indonesia

Received: 30 November 2024, Revised: 25 March 2025, Accepted: 9 April 2025, Published: 30 May 2025

Abstract

A survey of the incidence of lethal wilt disease in *Acacia mangium* plantations was conducted in three locations in South Sumatra, Indonesia: Ogan Komering Ilir (OKI) Regency, Ogan Ilir (OI) Regency, and Palembang City. Wilt disease in *Acacia* plants is highly concerning, with a mortality rate reaching 100%. Isolates were obtained from sapwood that exhibited dark spots, and six isolates were identified: CAW30658 (OKI), CAW30820, CAW30819, CAW31211, CAW30656 (OI), and CAW80912 (Palembang City). The initial symptom of wilting in *Acacia* leaves is characterized by leaf wilting, with color changes from green to yellow, followed by leaf drying. In the final stage, the tree dries out and dies. Symptoms in the sapwood include the formation of brown lesions that gradually turn black with elongated streak patterns resembling claw marks. These lesions also spread extensively into the heartwood, obstructing the plant's vascular tissues. Typically, infected trees emit a sweet, fruity odor from the exudate of fermenting lesions. This study aimed to identify the pathogen causing wilt in infected *A. mangium* using morphological characteristics and comparing DNA sequences of the Internal Transcribed Spacer (ITS) region and β-tubulin 1 (βt1) sequences, as well as its pathogenicity toward other host plants. The isolated fungus exhibited morphological characteristics similar to the wilt pathogen *Ceratocystis* sp., with isolates producing rounded ascomatal bases with long-necked ostiolar hyphae. Phylogenetic analysis confirmed *C. fimbriata,* differentiating it from all other *Ceratocystis* species. The six isolates showed a DNA similarity level of 98%. Koch's postulates test on four-month-old *A. mangium* confirmed that *C. fimbriata* was the causative agent of the wilt disease. The lowest pathogenicity test was on *Artocarpus heterophyllus*, with a rate of 6.11%, while the strongest attack was on *Annona muricata*, with a rate of 8.96% among other hosts.

Keywords: wilt disease; Fabaceae; morphological characteristics; DNA sequence; Ceratocystidaceae

*Corresponding author: E-mail: a_muslim@unsri.ac.id
https://doi.org/10.55003/cast.2025.265486

1. Introduction

Brown salwood (*Acacia mangium*) belongs to the Fabaceae family (Mitra et al., 2023). It was first introduced to northeastern Australia's tropical forests in 1966 and later to countries like Malaysia, Papua New Guinea, Indonesia, and Vietnam (Hardiyanto & Nambiar, 2014; Koutika & Richardson, 2019). *Acacia* plantations cover significant areas in Southeast Asia, with 1.1 million hectares in Vietnam alone (Harwood & Nambiar, 2014). *Acacia crassicarpa* was introduced to Thailand in the early 1980s and soon after to Indonesia, where it thrived in Sumatra's peatlands (Evans et al., 2019; Nasution et al., 2019).

Acacia is cultivated in Indonesia as a production forest species (Nasution et al., 2019). *Acacia mangium* offers numerous benefits in forestry ecosystems and agroforestry and is a preferred species for development due to its fast growth (Koutika & Richardson, 2019; Nair et al., 2021). This tree has a cylindrical trunk with grayish-brown bark (Karlinasari et al., 2021). *Acacia* has the ability to improve barren or infertile soils. Being a legume, it can fix nitrogen, which enhances soil quality, while its strong root system helps bind the soil and prevent erosion (Li et al., 2024). *Acacia* stem is used for producing briquettes (Souza et al., 2021) and paper (Mutiar et al., 2020). *Acacia* is valued for its strong and durable wood, making it a popular choice for construction and furniture (Amadou et al., 2020). Methanol extracts from *Acacia* leaves have demonstrated antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* (Rahman et al., 2014; Silva et al., 2016). Several species of *Acacia* contain bioactive compounds with antibacterial, anti-inflammatory, and antioxidant properties, which are used in traditional medicine (Adhikari & Rangra, 2023).

Acacia mangium is frequently attacked by pests and plant pathogens (Chauhan et al., 2020; Barnes et al., 2023), which hinders its growth and limits its development. Many pests such as the armyworm (*Spodoptera litura*) target the leaves (Sulistyono et al., 2020). Ginawan et al. (2019) reported that the nettle caterpillar (*Setora nitens*) completely perforates and consumes the leaves, while the tussock moth (*Orgyia leucostigma*) causes the leaves to dry out. Pests that attack the trunk include beetles like the ambrosia beetle (*Platypus trepanatus*), which bores holes into the trunk to release spores and fungal symbionts, leading to wilt and eventual death, and the rhinoceros beetle (*Oryctes rhinoceros*), which strips the bark with its pincers. The intensity of pest attacks on *Acacia* leaves is 39.6%, while the intensity of pest attacks on the trunk is 3.8%. Several diseases also affect *A. mangium*. Francis et al. (2014) reported that the growth and survival of *A. mangium* was increasingly threatened by red root rot disease caused by *Ganoderma philippii*.

From 2019 until now, cases of wilt disease, suspected to be caused by *Ceratocystis*, have been found in *Acacia* plants in South Sumatra. The initial symptoms include wilting leaves, followed by the appearance of black lesions on the sapwood, eventually leading to the drying out and death of the tree. This infestation has occurred in several plantations and wild plants across the regions of Ogan Ilir Regency, Ogan Komering Ilir Regency, and Palembang City. *Acacia* plants infected with *Ceratocystis manginecans* in Riau plantations exhibited symptoms such as the formation of lesions on the bark, which turned black and exuded gum-like sap, ultimately causing the plants to wilt and die (Tarigan et al., 2011). *Ceratocystis* sp. is a pathogen that causes wilt in several plant species including eucalyptus, duku, and *Acacia*. This pathogen can spread through soil, air, rainwater, and insects (Hughes et al., 2023). In eucalyptus, symptoms include sunken brown spots on the surface, which gradually spread, turn black, and cause the trunk to rot and dry out, leading to death (Pratama et al., 2023b). Muslim et al. (2022)

reported that duku trees infected with this pathogen developed yellowing, wilting, and drying leaves, eventually leading to the tree's death. Based on this background, the aim of this study was to investigate the characteristics and diversity of *Ceratocystis* sp. pathogens infecting *Acacia mangium*.

2. Materials and Methods

2.1 Disease distribution, sampling, and pathogen isolation

The distribution of the disease was observed in two regencies and one city: Ogan Ilir Regency (Pemulutan, Pulau Semambu, Tanjung Sejaro, and Sriwijaya University), Ogan Komering Ilir Regency (Kayu Agung), and Palembang City (Jakabaring), South Sumatra, Indonesia. The disease symptoms observed in the field included wilting and discoloration of leaves, the presence of wounds on the plants, the extent of lesion development in the sapwood tissue reaching the vascular tissue, the presence or absence of gum on the trunk, and the number of dead plants in the field. Observations were conducted five times: in May 2019, March 2020, February 2021, September 2022, and December 2023. Incidence was calculated using the following formula:

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total observed plants}}$$

The sample criteria were trees aged between 2 to 6 years. Samples were taken from sapwood and xylem tissue showing symptoms of brownish-black spots. The samples were collected by scraping off the tree's epidermal tissue using a large knife, approximately 5×10 cm in size. The samples were then wrapped in tissue paper, placed in ziplock bags to prevent contamination from other pathogens, and stored in a refrigerator (4°C) in the laboratory.

Wood samples from the field were cut into pieces of approximately 0.5×0.5 cm in size and surface sterilized by soaking in a 5% sodium hypochlorite (NaOCl) solution for 5 min, then rinsed with distilled water. Afterwards, they were soaked again in 70% alcohol for 5 min and rinsed with distilled water. The samples were then dried on filter paper inside a laminar airflow cabinet. Finally, the samples were inoculated onto malt extract agar (MEA) (Merck, Germany) and incubated at 25°C (room temperature).

2.2 Morphological characteristics

The characteristics of the isolates observed were collected from two regencies and one city where *Ceratocystis* was found: Ogan Ilir (Sriwijaya University; CAW30820, Tanjung Sejaro; CAW30819, Pemulutan; CAW31211, and Pulau Semambu; CAW30656), Ogan Komering Ilir (Kayu Agung; CAW30658), and Palembang (Jakabaring; CAW80912). The isolates were observed at 10 days old, cultured on MEA, and stored at 25°C (room temperature). Macroscopic morphology was assessed by examining colony color and mycelial growth patterns. Colony color determination was compared using the Munsell Soil Color Chart (Mueller et al., 2004). Each isolate was classified based on the colony's shape (circular, irregular, filamentous, rhizoid) and margin (entire, undulate, filiform, curled, lobate) as observed from mycelial growth. Microscopic observations were conducted by placing the isolate on a glass slide to examine and measure the shape of perithecia, conidia, spores, ascomata, conidiophores, and chlamydospores using an Olympus CX33

microscope, with the help of an Optilab Advance Plus and Image Raster 3 software. Each sample was measured 100 times. Morphological character data were analyzed using R-Studio. Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were applied to identify significant differences in the mean values of the morphological traits.

2.3 DNA isolation, PCR reactions, and sequence analysis

DNA was extracted from the pure cultures of six isolates collected from Ogan Ilir Regency (CAW30820, CAW30819, CAW31211, CAW30656), Ogan Komering Ilir Regency (CAW30658), and Palembang City (CAW80912). The isolates were propagated on potato dextrose broth (PDB) at an ambient temperature of 25°C for 12 days. The mycelia were then dried in an oven and ground with a mortar. DNA extraction was conducted using the YeaStar Genomic DNA Kit from Zymo Research Corporation. The concentration and purity of the DNA were measured using a NanoDrop ND-1000. To perform the polymerase chain reaction, the ITS and β-tubulin gene regions were amplified from the extracted DNA using the corresponding primer pairs, including βT1a 5′–(TCCGTAGGTGAACCTGCGG)–3′ and βT1b 5′–(GACGAGATCGTTCATGTTGAACTS)–3′ (Pratama et al., 2023a); ITS1 5′– (TCCGTAGG TGAACCTGCGG)–3′ and ITS4 5′–(TCCTCCGCTTATTGATATGC)–3′ (Pratama et al., 2021a). PCR was conducted using a C1000 Touch thermal cycler from Bio-Rad (USA). The PCR mixture comprised 12.5 µL of MyTaq HS Red Mix 2x from Meridian Bioscience (USA), 0.5 µL of each primer (10 mM), 9.5 µL of water, and 2 µL of DNA (2-10 ng). The thermal cycling program included an initial denaturation phase at 95°C for 1 min, followed by 35 cycles (95°C for 15 s, 55°C for 15 s, and 72°C for 10 s). The target amplification was completed with a last elongation step at 72°C for 10 min, and the amplification products were stored at 10°C. The PCR products were separated on a 1% agarose gel through electrophoresis at 95 volts for 25 min. A 100 bp DNA ladder (Geneaid) was used to estimate DNA size. The gel was stained with a Florosafe DNA Stain, and the results were visualized using a GelDoc Go Imaging System (Bio-Rad). The PCR products were sequenced by 1st Base Malaysia, and the resulting DNA sequences were compared to the GenBank database using a nucleotide BLAST search at the National Center for Biotechnology Information (NCBI). Relevant sequences were further processed and analyzed using BioEdit software (Pratama et al., 2021b).

Sequences of *Ceratocystis* species related to *Acacia mangium* were obtained from GenBank. Phylogenetic sequences from various gene regions were aligned using Mesquite v3.5 and manually refined. A phylogenetic tree, based on a combined ITS and β-tubulin dataset, was generated and analyzed as a single dataset. Maximum Parsimony (MP) analysis, with 1,000 bootstrap replications, was conducted in MEGA v11 to evaluate the relationship (Kumar et al., 2016; Pratama, et al., 2023c).

2.4 Koch's postulate test and host range

Koch's postulates were tested on six-month-old *A. mangium,* and host range experiments were conducted using seedlings of six-month-old *Artocarpus heterophyllus,* eight-month-old *Annona muricata,* two-year-old *Lansium domesticum,* and eight-month-old *Mimusops elengi.* The four plants are popular forest plantation species in South Sumatra and have been reported as plants infected with *Ceratocystis* disease in the field. The plants had 2-3 cm stem diameters and heights under 1 m. Pathogenicity was assessed using the under-bark inoculation method by Deidda et al. (2016). A 4 mm cork borer was used to expose

the cambium, and agar discs with mycelium from 2-week-old cultures on 2% MEA (Muslim et al., 2022) were placed on the wounds**.** *Ceratocystis* isolates were positioned with the mycelium facing the cambium. Ten seedlings per species were inoculated, with sterile MEA plugs as controls. Masking tape covered the inoculation sites, and polyethylene film sealed the log end to prevent desiccation and contamination.

Each row plot contained ten replicate plants per treatment. After 45 days, lesion lengths and death percentages were recorded. Wood samples were taken from lesions beyond the inoculation sites, and the pathogen was reisolated and sequenced to confirm Koch**'**s postulates. Pathogenicity data were analyzed using R**-**Studio. Analysis of variance (ANOVA) and Tukey**'**s HSD test were used to determine significant differences between treatment means.

3. Results and Discussion

3.1 Disease distribution, sampling, and pathogen isolation

The wilt disease affecting *Acacia* in South Sumatra was spread across two districts and one city: Ogan Komering Ilir (OKI), Ogan Ilir (OI), and Palembang (see Table 1). In OKI, the disease was found in one location, with an incidence rate ranging from 25% to 63.15% from 2019 to 2023, representing an increase of 38.15%. In OI, the disease occurred in four locations, with the highest incidence at the University of Sriwijaya, reaching 100%, and the lowest at Pulau Semambu, at 57.4%. Each location showed an increase, with the University of Sriwijaya at 16.7%, Tanjung Sejaro at 53.1%, Pemulutan at 44.1%, and Pulau Semambu at 20.37%. In Palembang, the disease was observed at one point in Jakabaring, with an incidence of 29.03% to 87.1%, representing an increase of 58.07%.

Table 1. Incidence of *Acacia mangium* wilt disease in South Sumatra

Location (∑ Trees)	Incidence (%)				
	May 2019	March 2020	February 2021	September 2022	December 2023
OKI Kayu Agung (n=76)	25	32.89	39.47	52.63	63.15
OI UniversitsSriwijaya (n=120)	83.3	95.8	100	100	100
TanjungSejaro (n=32)	31.3	37.5	59.4	68.8	84.4
Pemulutan (n=34)	14.7	26.5	35.3	41.2	58.8
PulauSemambu (n=54)	37.03	40.7	46.3	51.9	57.4
Palembang Jakabaring (n=31)	29.03	38.7	64.5	80.6	87.1

The wilt disease affecting *Acacia mangium* in South Sumatra since 2019 has caused many plants to die suddenly (Figure 1a). The disease is characterized by early symptoms of wilting leaves, which change color from green to yellow and eventually become dry (Figure 1b). As the disease progresses, the trees wither and die. Internal symptoms include brown lesions on the bark that gradually turn black and take on an elongated, scratch-like appearance (Figure 1c). In the sapwood of the affected plants, numerous holes made by Nitidulidae insects can be observed (Figure 1d). Generally, affected plants produce gum on their stems (Figure 1e). This outbreak was reported in two districts and one city: Ogan Ilir, Ogan Komering Ilir, and Palembang.

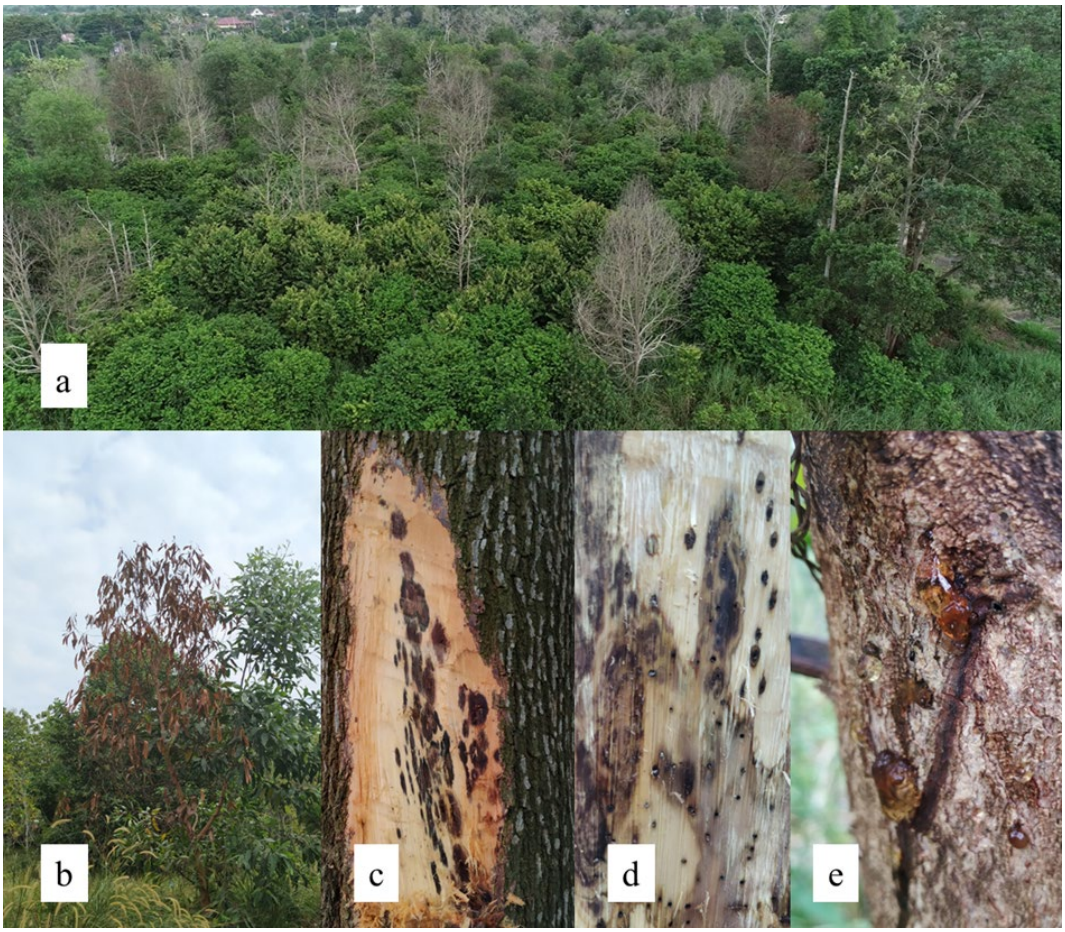

Figure 1. Symptoms of the disease caused by *Ceratocystis fimbriata* on *Acacia mangium* in South Sumatra. a. Dead and affected trees, b. *Acacia mangium* (dried and partially died), c. Lesions on the sapwood, d. Holes in the bark caused by Nitidulidae insects, e. Gum on the stem of *A. mangium* affected by the disease

A similar disease affecting *Acacia mangium* was first reported in Indonesia in several plantation areas, including Teso, Logas, and Pelalawan, all located in Riau (Tarigan et al., 2011). The symptoms observed include changes in the bark and wood of the acacia around the cankers, which turned black due to the exudation of sap. The

discolored wood typically displayed striped patterns, transitioning evenly from dark brown to deep blue as it aged. Similar symptoms have been identified in South Sumatra, with the first report occurring in 2019. This pathogen has been detected in several locations across South Sumatra, such as Ogan Komering Ilir (Kayu Agung), Ogan Ilir (Universitas Sriwijaya, Tanjung Sejaro, Pemulutan, and Pulau Semambu), and the city of Palembang (Jakabaring). Infection levels varied across locations, with Universitas Sriwijaya recording the highest rate at 100%, marking a 16.7% increase. Palembang showed the most notable surge, reaching 58.07%, followed by Tanjung Sejaro at 53.1%. Pemulutan reported a 44.1% rise, while Kayu Agung and Pulau Semambu recorded increases of 38.15% and 20.37%, respectively. All locations are close to one another, allowing the pathogens such as fungi to spread more easily to nearby hosts through mediums like wind, water, insects, or physical contact. The pathogen's spread follows a dispersal gradient or disease gradient, where the concentration of inoculum and disease intensity decreases with increasing distance from the source of inoculum (Saharan et al., 2016; Ojwang' et al., 2021).

The distance from an inoculum source influences the severity of the disease (Cardillo et al., 2018; González-Domínguez et al., 2020). There is an exponential relationship between the distance to the inoculum source and the incidence of the disease; the closer the inoculum source, the higher the incidence, and vice versa (Jeger & Termorshuizen, 2017; Smith & Casadevall, 2022). *Ceratocystis fimbriata* is a soil-borne pathogen that can spread through the soil (Baccelli et al., 2014). Additionally, trees that are wounded due to human activities or other mammals can enhance the spread of the *C. fimbriata* pathogen, leading to wilt disease (Brawner et al., 2015). *Hypocryphalus mangiferae* and rodents play a significant role in the dissemination of this pathogen, as they can spread it by biting infected stems and then transferring it to healthy ones (Chi et al., 2019; Muslim et al., 2022; 2025). The increase in disease observed at the monitoring sites can be significantly attributed to various factors that facilitate the wider spread of this pathogen. Plant resistance to a fungal pathogen is influenced by genetic factors, chemical resistance, and structural resistance (Lin et al., 2021; Nazarov et al., 2020).

3.2 Morphological characteristics

The characteristics of the observed isolates are shown in Figure 2. The isolates CAW30820, CAW30819, CAW31211, and CAW30656 were representive of the Ogan Ilir Regency, isolate CAW30658 was representative of the Ogan Komering Ilir Regency, and isolate CAW80912 was representative of the city of Palembang.

All fungal isolates cultured on MEA medium exhibited relatively similar colors, predominantly olive gray, except for isolate CAW30819, which displayed an olive color. All isolates showed moderate mycelial growth patterns, characterized by irregular shapes and undulate margins (Table 2).

All isolates obtained exhibited black ascomata with bases ranging from globose to subglobose (Figures 3a1-3a6), and they featured black ascomatal necks that contained ostiolar hyphae at their tips, which were divergent and transparent (Figures 3b1-b6). All isolates possessed clamped, hyaline chlamydospores (Figures 3c1-c6), phialides (Figures 3d1-d6), and ascospores that were both hat-shaped and without a cap (Figures 3e1-3e6). Each isolate had cylindrical conidia (Figures 3f1-f6). The measurements of the *Ceratocystis fimbriata* isolates showed consistent sizes. Measurements were taken from the ascomatal

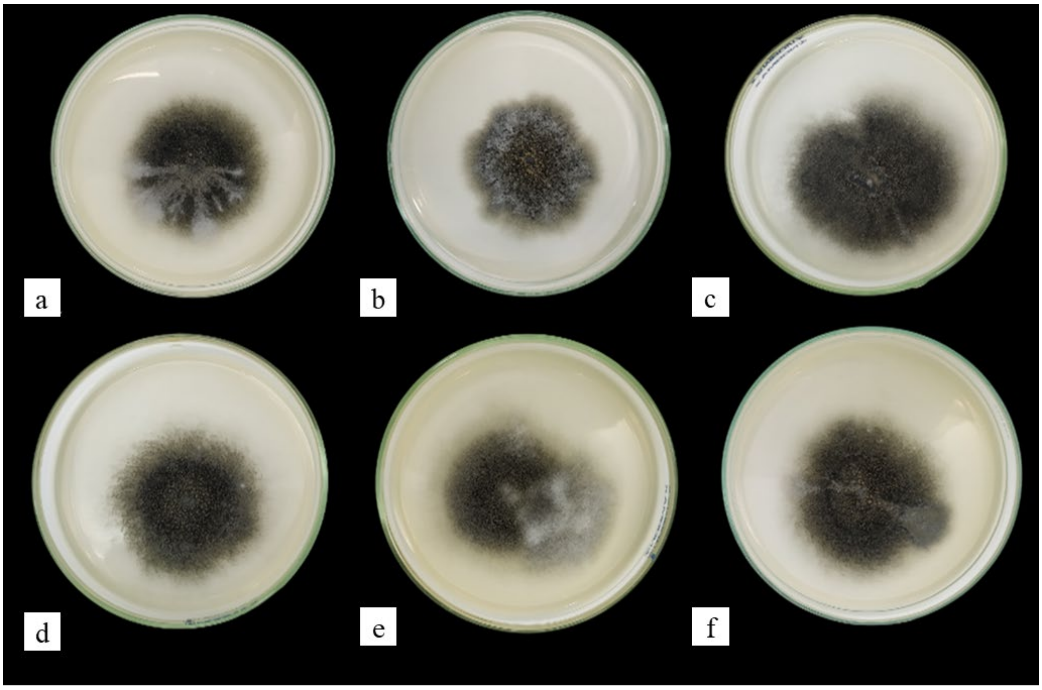

Figure 2. Colony morphology of *Ceratocystis fimbriata* isolates on MEA medium. a. CAW30820, b. CAW30819, c. CAW31211, d. CAW30656, e. CAW30658, f. CAW80912

Table 2. Characteristics of *Ceratocystis fimbriata* fungal isolates on MSA media

Isolate Origin (Code)	MEA Media				
	Colony		Mycelium Growth Pattern	Form	Margin
	Color	Code			
Universitas Sriwijaya (CAW30820)	Olive gray	5Y;4/2	Moderate	Irregular	Undulate
Tanjung Sejaro (CAW30819)	Olive	5Y;4/3	Moderate	Irregular	Undulate
Pemulutan (CAW31211)	Olive gray	5Y;4/2	Moderate	Irregular	Undulate
Pulau Semambu (CAW30656)	Olive gray	5Y;4/2	Moderate	Irregular	Undulate
KayuAgung (CAW30658)	Olive gray	5Y;4/2	Moderate	Irregular	Undulate
Jakabaring (CAW80912)	Olive gray	5Y;4/2	Moderate	Irregular	Undulate

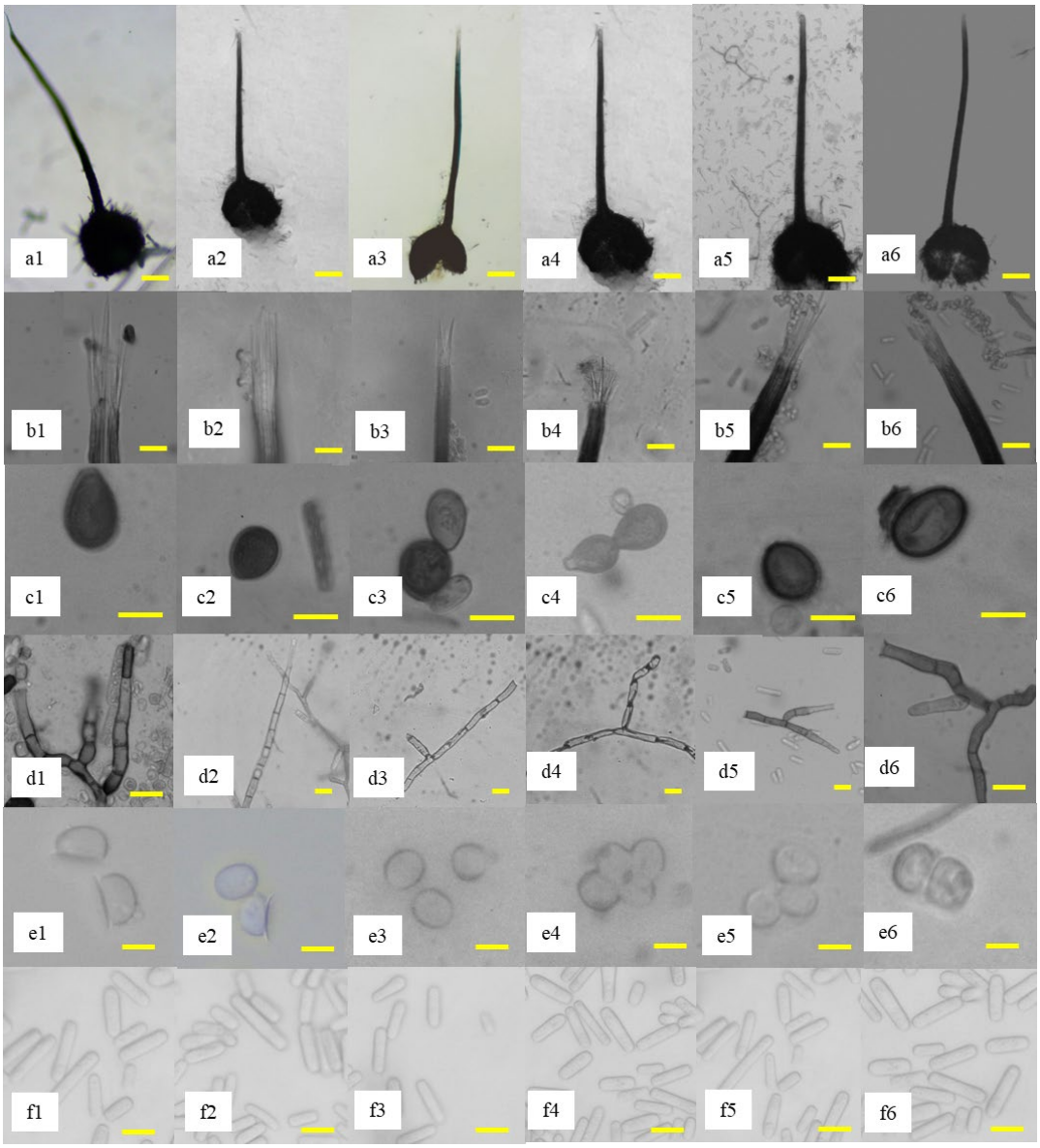

Figure 3. *Ceratocystis fimbriata* morphology. a. Perithecial-shaped ascomata, b. Divergent ostiolar hyphae, c. Chlamydospores, d. Conidiophore/phialide, e. Hat-shaped ascospores, f. Cylindrical conidia. 1. CAW30820; 2. CAW30819; 3. CAW31211; 4. CAW30656; 5. CAW30658; 6. CAW80912. Scale bars: a = 100 µm; b, c, e, f = 5 µm; d = 5 µm

base, ascomatal neck, ostiolar hyphae, ascospores, and chlamydospores. The ascomatal base width ranged from 131.96±35.74 to 189.09±49.17, and the length measured between 138.54±25.44 and 189.40±56.66. The ascomatal neck length ranged from 351.43±108.46 to 467.18±11.34, with a top width of 9.70±1.69 to 18.18±3.95 and a bottom width of 20.86±4.6 to 30.63±6.58. The length of the ostiolar hyphae measured between

38.13±12.76 and 48.97±8.24. Hat-shaped ascospores measured between 5.04±0.82 and 3.04±0.58 in length. Ascospores without a sheath had a width of 4.92±0.87 to 2.46±0.45, while those with a sheath measured 5.73±1.02 to 4.12±0.77. The primary conidia length ranged from 17.47±3.79 to 11.99±2.43, and their width measured between 7.45±1.69 and 3.54±0.68. Secondary conidia lengths ranged from 30.95±7.15 to 6.64±1.55, and their widths from 8.16±1.25 to 3.94±0.80. Chlamydospore lengths ranged from 12.30±2.49 to 8.87±1.67, and widths ranged from 12.12±2.24 to 8.71±1.52 (Table 3).

Identification of the pathogen based on morphology indicated that the isolates belong to the genus *Ceratocystis*, and confirmation of the species through DNA sequence comparison identified it as *C. fimbriata*. The isolates obtained from Ogan Ilir (OI), Ogan Komering Ilir (OKI), and Palembang all exhibited perithecia with a black color and a base resembling a globe. The dimensions, shape, and color of these isolates were not significantly different from those of *C. fimbriata* found on *Acacia mangium* from Malaysia (Syazwan et al., 2021). However, when comparing the sizes, the isolates from OI, OKI, and Palembang were slightly smaller than the *C. fimbriata* isolates found on *Eucalyptus* from Australia (Nkuekam et al., 2013). *Ceratocystis fimbriata* sourced from *Hevea brasiliensis* in Brazil (Valdetaro et al., 2015) was found to be slightly larger than the isolates from OI, OKI, and Palembang. The size variations of *C. fimbriata* isolates are influenced by the host and climate; isolates in subtropical climates tend to be slightly larger than those in tropical climates.

3.3 DNA isolation, PCR reactions, and sequence analysis

The PCR amplification of the ITS and β-tubulin gene regions resulted in fragments of approximately 550 base pairs in size. The resulting sequences were stored in the GenBank database and compared with other *Ceratocystis* sequences (Table 4). A BLAST search of the β-tubulin gene showed that the *C. fimbriata* sensu stricto isolates had a 99% sequence identity. Additionally, analysis of the ITS gene data revealed that the isolates were primarily represented by ITS5, which shared 100% similarity with CAL32195, previously isolated from the duku plant, the original source of the disease (Figure 4). The phylogenetic relationships between the selected isolates and related taxa were determined using the Maximum Parsimony (MP) method with 1,000 replications, combining the ITS and β-tubulin sequences. The results indicated that *C. fimbriata* isolates from *A. mangium* were closely related to those from *Lansium domesticum* in Indonesia. This sequence similarity, along with phenotypic characteristics, confirmed that the causative agent of wilt disease in *A. mangium* in South Sumatera was classified as *C. fimbriata*.

Separate analysis based on ITS sequences identified two haplotypes, ITS5 and ITS7, from *C. fimbriata* sensu stricto, and the use of β-tubulin placed them in the Latin American Clade (LAC) of *C. fimbriata* sensu lato (not published). The comparative DNA analysis of combined ITS and β-tubulin sequences from six isolates indicated that *C. fimbriata* was responsible for wilt disease in *A. mangium* in South Sumatra. All six isolates were found to be identical to the sequences of *C. fimbriata* from isolate CAW80912, which originated from *Lansium domesticum* (Muslim et al., 2022). *Acacia mangium* is recognized as a principal tree species for industrial tree plantations and reforestation in Indonesia. Therefore, the *C. fimbriata* pathogen should be investigated and monitored in *A. mangium* plantations. Further research on the management of this pathogen in *A. mangium* is essential to protect commercial plantations.

Table 3. Morphology of *Ceratocystis fimbriata* isolates of *Acacia mangium* from different districts in South Sumatra

Morphological Characters	Isolates					
	CAW30820	CAW30819	CAW31211	CAW30656	CAW30658	CAW80912
Ascomatal bases						
Shape						
Ascomatal base (W)	166.13±27.84^{c}	139.88±37.29^{ab}	168.31±60,70^{c}	131.96±35.74^{a}	189.09±49,17^{d}	159.06±46.92^{bc}
Ascomatal base (L)	170.77±27.47^{bc}	138.54±25.44^{a}	181.54±60.35^{c}	148.34±47^{a}	189.40±56.66^{c}	158.17±45.17^{ab}
Ascomatal neck						
Neck (L)	467.18±110.34^{c}	410.84±96.26^{b}	371.14±96.56^{ab}	351.43±108.46^{a}	397.11±98.73^{ab}	416.02±79.17^{b}
Neck (W) top	18.18±3.95^{c}	10.91±2.68^{ab}	11.98±3.08^{b}	11.33±2.505^{b}	11.89±2.55^{b}	9.70±1.69^{a}
Neck (W) bottom	30.40±5.6^{c}	22.77±5.84^{a}	30.63±6.58^{c}	26.41±5.34^{b}	26.16±6.16^{b}	20.86±4.68^{a}
Ostiolar hyphae						
Shape						
Ostiolar hyphae (L)	38.13±12.76^{a}	40.58±9.62^{ab}	47.09±8.91^{c}	44.31±7.42b^{c}	48.97±8.24^{c}	47.25±13.13^{c}
Ascospores						
Hat-shaped ascospores (L)	3.96±0.98^{c}	3.17±0.83^{ab}	3.45±0.43^{b}	3.04±0.58^{a}	4.19±0.62^{c}	5.04±0.82^{d}
Ascospores (W)	4.92±0.87^{d}	2.47±0.39^{a}	3.45±0.48^{b}	2.46±0.45^{a}	3.55±0.52^{b}	4.63±0.79^{c}
without sheath	4.57±0.86^{bc}	5.73±1.02^{d}	4.55±0,78^{bc}	4.47±0.65^{ab}	4.88±0.61^{c}	4.12±0.77^{a}
Ascospores (W) with sheath	12.22±3.58^{a}	11.99±2.43^{a}	13.12±2.80^{a}	12.87±2.75^{a}	12.24±3.38^{a}	17.47±3.79^{b}
Primary conidia (L)	4.44±1.11^{b}	3.94±0.67^{a}	3.54±0.68^{a}	4.00±0.60^{ab}	5.02±1.19^{c}	7.45±1.69^{d}
Primary conidia (W)	10.82±2.59^{b}	16.34±6.37^{c}	30.95±7.15^{d}	11.46±3.71^{b}	11.38±3.97^{b}	6.64±1.55^{a}
Secondary conidia (L)	3.94±0.80^{a}	5.62±2.01^{c}	8.16±1.25^{d}	4.78±0.84^{b}	4.88±0.91^{b}	5.09±1.03^{bc}
Secondary conidia (W)						
Chlamydospores						
Shape	11.48±1.91^{bc}	8.87±1.67^{a}	12.47±2.07^{d}	12.30±2.49^{cd}	11.05±1.99^{b}	11.67±2.16^{bcd}
Chlamydospores (L)	11.03±1.89^{b}	8.71±1.52^{a}	11.04±8.04^{b}	11.01±2.38^{b}	11.49±2.46^{b}	12.12±2.24^{b}

Table 4. Details of collection and GenBank accession numbers for ITS and β-tubulin sequences of *Ceratocystis fimbriata* isolates analyzed in this study.

Isolate No.	*Ceratocytis* Species	Host	Origin	Collector	GenBank Accession Isolate	
					ITS	βT
CAW30820	*C. fimbriata*	*Acacia mangium*	Indonesia	A. Muslim	MT374247	Submitted
CAW30912	*C. fimbriata*	*Acacia mangium*	Indonesia	A. Muslim	MT374246	Submitted
CAW30819	*C. fimbriata*	*Acacia mangium*	Indonesia	A. Muslim	MT374245	Submitted
CAW31211	*C. fimbriata*	*Acacia mangium*	Indonesia	A. Muslim	MT374244	Submitted
CAW30656	*C. fimbriata*	*Acacia mangium*	Indonesia	A. Muslim	MT374243	Submitted
CAW30658	*C. fimbriata*	*Acacia mangium*	Indonesia	A. Muslim	MT374243	Submitted
CAL32195	*C. fimbriata*	*Lansium domesticum*	Indonesia	A. Muslim	MT373416.1	MW752145
CMW22581	*C. manginecans*	*Acacia mangium*	Indonesia	M. Tarigan	EU588658	EU588638
CAL32156	*C. fimbriata*	*Lansium domesticum*	Indonesia	A. Muslim	MT373422.1	MW752143.1
CMW22579	*C. manginecans*	*Acacia mangium*	Indonesia	M. Tarigan	EU588659	EU604671
CMW4068	*C. albifundus*	*A. mearnsii*	RSA	J. Roux	DQ520638	EF070429
CMW14793	*C. caryae*	*C. cordiformis*	U.S.A	J. Johnson	EF070424	EF070439
CMW14800	*C. smalleyi*	*C. cordiformis*	U.S.A	G. Smalley	EF070420	EF070436
CMW14789	*C. populicola*	*Populus* sp.	Poland	J. Gremmen	EF070418	EF070434
CMW19385	*C. atrox*	*E. grandis*	Australia	M.J. Wingfield	EF070415	EF070431
CMW23808	*C. obpyriformis*	*A. mearnsii*	South Africa	R.N. Heath	EU245003	EU244975
CMW23808	*C. obpyriformis*	*A. mearnsii*	South Africa	R.N. Heath	EU245003	EU244975
CMW22579	*C. manginecans*	*Acacia mangium*	Indonesia	M. Tarigan	EU588658	EU588638

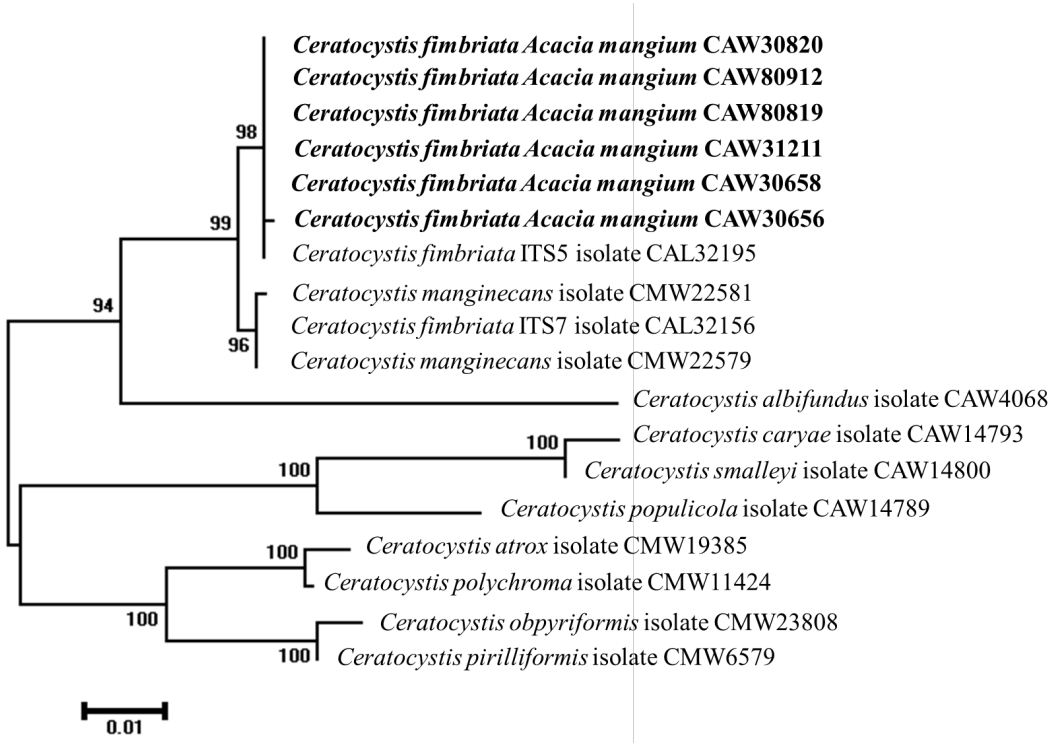

Figure 4. Phylogenetic relationships of *Ceratocystis* isolates from a dataset of ITS and β-tubulin regions sequences alignment. The phylogram was generated through a heuristic search based on parsimony, with isolates representing *Ceratocystis fimbriata* from *Acacia mangium* in Soth Sumatra highlighted.

3.4 Koch's postulate and host range test

Acacia mangium seedlings inoculated under Koch's postulates exhibited discoloration in the bundle vessels and wilting of the leaves. ANOVA revealed significant differences in lesion lengths among all isolates inoculated on this host. Lesion lengths in the inoculated *A. mangium* seedlings ranged from 6.04 to 11.2 cm (Figure 5). Statistical analysis revealed a significant difference in lesion length between inoculated seedlings and controls. *Ceratocystis fimbriata* was successfully re-isolated from inoculated seedlings, whereas no fungus was found in the control group. The *C. fimbriata* isolates inoculated on other test seedlings caused death and significant lesions. Based on seedling mortality percentages, the severity was classified as severe or moderate. In *A. muricata* and *M. elengi* seedlings, all isolates caused severe pathogenic symptoms, with lesion lengths of 6.03-12.58 cm and 6.06-11.14 cm, respectively. In contrast, *A. heterophyllus* and *L. domesticum* seedlings exhibited moderate symptoms, with lesion lengths of 5.76-8.33 cm and 6.12-14.76 cm, respectively, compared to controls that averaged a lesion length of only 0.1 cm from the inoculation scar (Figure 5).

The percentage of seedling mortality in the host range trials had different percentages (Figure 6). Isolates caused severe attacks with the percentages of *A.*

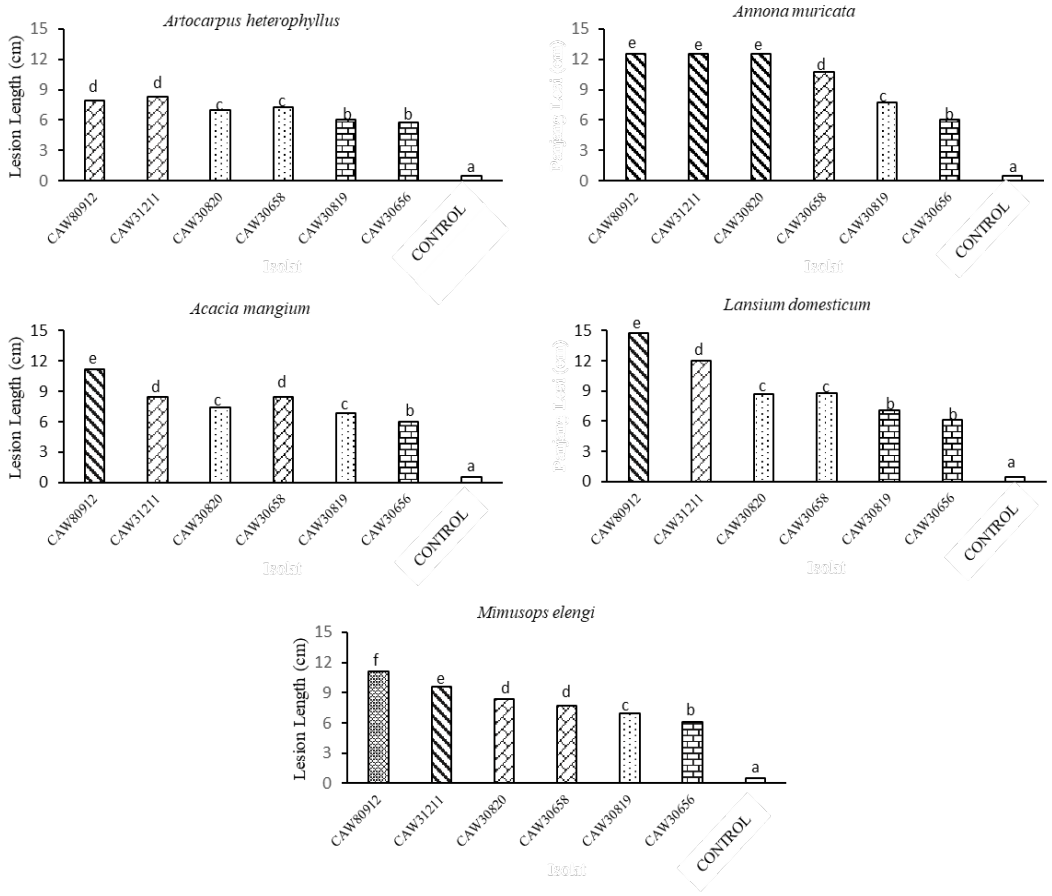

Figure 5. Lesion length from pathogenicity test of *Ceratocystis fimbriata* isolates on *Acacia mangium, Artocarpus heterophyllus, Annona muricata, Lansium domesticum,* and *Mimusops elengi* seedlings. Plants have 2-3 cm stem diameters and heights <1 m. Notes: Tukey's HSD multiple range test shows that isolates sharing the same letters in a column do not exhibit significant differences at a significance level of P=0.05.

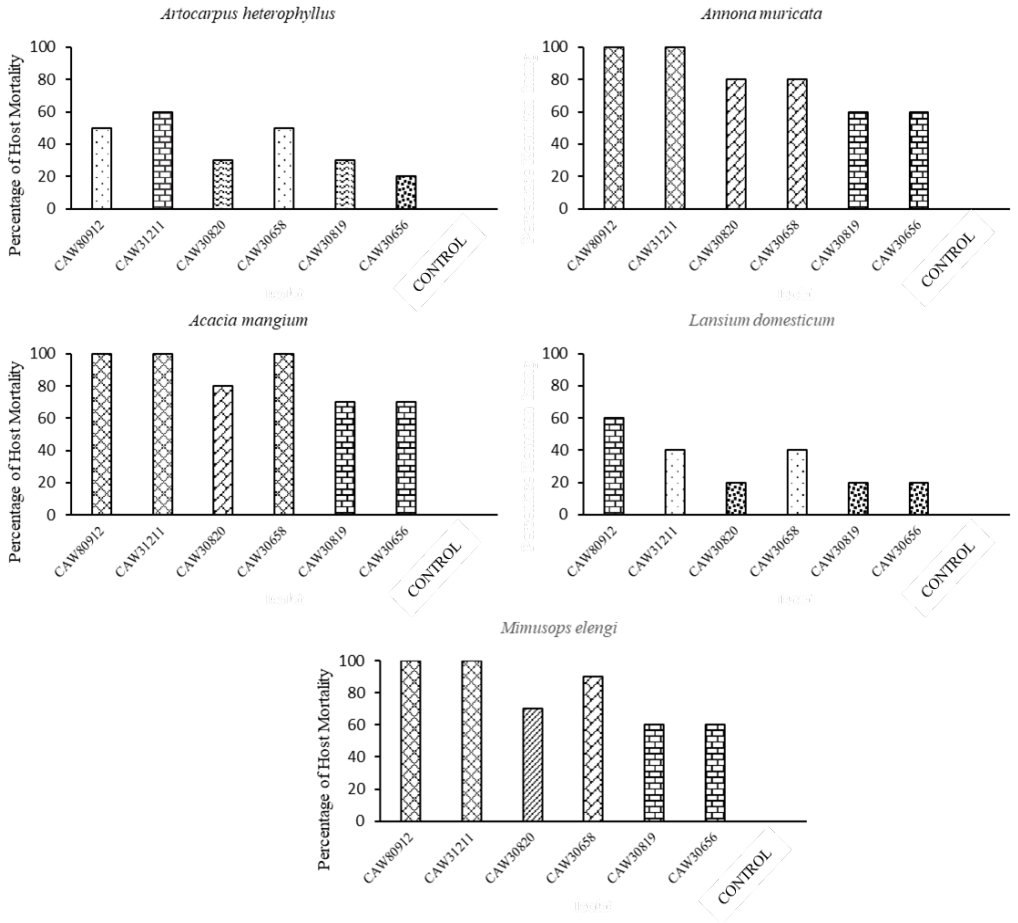

Figure 6. Percentage of host mortality from pathogenicity test of *Ceratocystis fimbriata* isolates on *Acacia mangium, Artocarpus heterophyllus, Annona muricata, Lansium domesticum,* and *Mimusops elengi* seedlings after 30-45 days post-inoculation.

mangium, A. muricata, and *M. elengi* seedling mortality being 70%-100%, 60%-100%, and 60%-100% at 45 days, and all seedlings died at 70 days after pathogen inoculation, respectively. In *A. heterophyllus* and *L. domesticum* seedlings, these isolates caused moderate symptoms with the percentages of seedlings mortality being 20%-50 % and 46%-82% at 45 days, respectively. No dead control seedlings were found.

Acacia mangium is the primary host of *C. fimbriata* because it is one of the agroforestry plants. Muslim et al. (2022) conducted a study using *C. fimbriata* isolates derived from duku plants, which were inoculated into *A. mangium*, resulting in a 100% mortality rate and lesion lengths ranging from 9.94 to 20.93 cm. In a study by Tarigan et al. (2011) on *A. mangium*, the lesion lengths ranged from 1.4 to 14.1 cm. *Acacia mangium* is particularly vulnerable to attacks by *C. fimbriata*, which can cause wilt disease (Brawner et al., 2015). Research by Suwandi et al. (2021) indicated that *A. mangium* was more susceptible than duku plants, with duku being the primary host in their study. The pathogen *C. fimbriata* exhibited varying levels of aggressiveness on different host plants (Oliveira et

al., 2015; Fernandes et al., 2022). The longest lesions on jackfruit plants were found for isolate CAW31211, measuring 8.33 cm, while the shortest lesions measured 5.76 cm. For soursop plants, the longest lesion was 12.58 cm from isolate CAW31211, and the shortest was 6.03 cm from isolate CAW30656. The *C. fimbriata* isolate from *A. mangium* CAW80912 showed the longest lesion at 11.20 cm, while the shortest lesion was 6.04 cm. The host range test results indicated that the longest lesions originated from soursop (isolate CAW31211) at 12.58 cm, while the shortest lesions came from jackfruit (isolate CAW30819) at 5.76 cm. *C. fimbriata* is known to attack agroforestry plants (Pratama et al., 2021a; Pratama et al., 2025). The mortality rate of *A. heterophyllus* is relatively low and it is quite resistant to *Ceratocystis* disease, so this plant has the potential to be developed in areas that are severely affected by *Ceratocytis* disease to control the wider spread of this disease.

4. Conclusions

The cause of wilt and sudden death disease in *A. mangium* plants in South Sumatra was confirmed through morphological characteristics, Koch's postulates, and molecular analysis, to be *C. fimbriata*. This pathogen is known to have a wide range of host plants. The identification and pathogenicity studies of the pathogen provide very useful as initial data that is essential to control this disease.

5. Acknowledgements

This work was financially supported by National Competitive Research from Kemdikbudristek 2024 SP 090/E5/PG.02.00.PL/2024 on June 11, 2024, by the Rector's Decree Number 0459/E5/PG.02.00/2024 on Mei 30, 2024

6. Authors' Contributions

RP performed the collection, assembly of data, and the critical revision of the article. AM, S, ND, and HH performed research concept and design, data analysis and interpretation, writing the manuscript, and final approval. RPA prepared and performed morphological and molecular identification.

7. Conflicts of Interest

No Potential conflict of interest relevant to this article was reported.

ORCID
Rizki Putri Amelia https://orcid.org/0009-0007-9084-8556
A. Muslim https://orcid.org/0000-0002-3973-7443
Nurhayati Damiri https://orcid.org/0000-0002-9289-1079
Harman Hamidson https://orcid.org/0009-0002-4038-9957
Suwandi https://orcid.org/0000-0003-3096-5797
Rahmat Pratama https://orcid.org/0000-0002-7967-8505

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