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Ceratocystis fimbriata on Brown Salwood (*Acacia Mangium*) in Banyuasin, Indonesia

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ABSTRACT

Acacia mangium in Banyuasin commonly grows in plantations and along roadsides as a shade plant. Generally, this plant is also useful as a material for making household furniture, firewood, and paper pulp. Since 2022, numerous reports of these plants wilting and dying suddenly, have drawn significant attention in the plantations. Our study recorded that the presence of cankers on the stems, lesions on the sapwood, blackened vascular tissue, discoloration of leaves, wilting of the canopy, and massive tree mortality characterize this disease. Based on the morphological characteristics and sequencing of the ITS and β -tubulin genes, the pathogen causing canker and wilt disease in *A. mangium* in Banyuasin has been confirmed as *Ceratocystis fimbriata*. The pathogen strain exhibits strong pathogenicity on *A. mangium* seedlings during inoculation tests in nurseries. Host range tests showed that the isolate can also thrive on *Acacia crassicarpa* and jackfruit (*Artocarpus heterophyllus*). Therefore, it is crucial to identify appropriate management solutions to minimize damage to *A. mangium* plants.

KEYWORDS: *Ceratocystis fimbriata*, *Acacia mangium*, Stem canker, Wilt diseases

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INTRODUCTION

Brown Salwood, or *Acacia mangium*, is a species cultivated in industrial forest plantations (HTI) in Sumatra, including South Sumatra (Nurrohmah *et al.*, 2020). HTI in Indonesia covers an area of approximately 3.8 million hectares (Greenhill *et al.*, 2017), with 296,400 hectares located in South Sumatra (Maryadi *et al.*, 2019). In addition to Indonesia, *Acacia* is widely planted in Southeast Asia, with plantation areas estimated at 20,000 hectares in Thailand, 49,000 hectares in Malaysia, 50,000 hectares in China, 800,000 hectares in Indonesia, and 1.1 million hectares in Vietnam (Harwood & Nambiar, 2014). *A. mangium* originates from Indonesia, Papua New Guinea, and Australia (Koutika & Richardson, 2019) and belongs to the family Fabaceae. In tropical climates, this plant can grow to a height of up to 30 meters (Jusoh *et al.*, 2014).

A. mangium grows rapidly and demonstrates superior growth compared to other species under normal conditions. It also adapts well to stressed environments, such as sandy soils with low fertility, making it suitable for reforestation and land reclamation projects (Asif *et al.*, 2017). *A. mangium* can fix

atmospheric nitrogen through a symbiotic relationship with bacteria in its root nodules. This ability enhances soil fertility by increasing nitrogen content (Hamad-Sheip *et al.*, 2021). This tree species has significant economic value (Suharti & Widiarti, 2005). *Acacia* wood is widely utilized in industrial forest plantations to produce a variety of products, such as pulp (Cochard *et al.*, 2021), materials for furniture production (Tham *et al.*, 2021), and briquettes (Wijayanti *et al.*, 2021). Beyond furniture production, methanol extracts from acacia leaves have antibacterial properties effective against *Staphylococcus aureus* and *Escherichia coli* (Mudzenge *et al.*, 2017; Adhikari & Rangra, 2023).

The presence of pests and diseases is one of the factors contributing to decreased production and quality in industrial forest plantations, particularly in *Acacia* crops (Wingfield *et al.*, 2011). Several diseases affecting *A. mangium* include root diseases, leaf and shoot diseases, and wilt disease (Hurley *et al.*, 2023). In addition to diseases, various pests attack *A. mangium*, such as species from the family Hymenoptera: *Apidae*, *Aleyrodidae* (Hemiptera), *Phenacoccus* sp. (Hemiptera: Pseudococcidae), and *Aethalion reticulatum* L. (Hemiptera:

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Aethalionidae) (Demolin-Leite, 2023). Wilt disease caused by the pathogen *Ceratocystis fimbriata* is a significant threat to *A. mangium*. The incidence of *C. fimbriata* attacks in Malaysia ranges from 7.5% to 13.6% (Syazwan et al., 2021). Symptoms caused by *C. fimbriata* infection include lesions on the sapwood and the appearance of exudates (Yunus et al., 2024).

The disease caused by *Ceratocystis* in acacia plants is a serious threat, as it can reduce quality and lead to the death of the plants. In Sabah, Malaysia, the disease affecting acacia plants is caused by the pathogen *Ceratocystis fimbriata* (Syazwan et al., 2021). In Indonesia, *Ceratocystis manginecans* and *Ceratocystis acaciivora* are known to cause sudden wilt and death in *A. mangium* plants. In addition to *Acacia*, *C. fimbriata* also affects other plants, such as *Lansium domesticum* (Muslim et al., 2022), wilt disease of soursop (Pratama et al., 2023a), mango (Al Adawi et al., 2013, 2014), mahogany (Muslim et al., 2025), and eucalyptus (van Wyk et al., 2011). This pathogen can infect through soil (soil-borne) and water (water-borne) (Harrington, 2013). The wilt disease caused by *Ceratocystis fimbriata* shows symptoms such as sudden wilting of leaves, the appearance of black or gray lesions on the outer bark, and black streaks in the vascular tissue. These symptoms are followed by leaf yellowing, wilting, and eventual death of the plant (Tarigan et al., 2011). Research on *Ceratocystis* disease in *A. mangium* plants in Banyuasin has not been reported. This study aims to identify the cause of sudden wilt disease in *A. mangium* plants in Banyuasin through morphological and molecular identification and to evaluate the pathogenicity of the pathogen on other host plants.

MATERIALS AND METHODS

Sampling and Isolation of Pathogens

Observations were conducted at several locations in Banyuasin Regency where *A. mangium* plants are commonly found, including Pangkalan Balai, Sungai Pinang, Rambutan, and Sukajadi. The plants were examined for disease incidence, intensity, and symptoms in the field. Samples from diseased plants or those showing wilting symptoms were collected from the sapwood, where black or gray lesions were observed, measuring 5x10 cm. The collected samples were labeled, transported, and stored at the Plant Pathology Laboratory, Universitas Sriwijaya. The sapwood of diseased plants was surface-sterilized by immersing it in a sodium hypochlorite (NaOCl) solution for 5 minutes and then drying it on filter paper. Subsequently, the samples were soaked in alcohol for another 5 minutes, rinsed with distilled water (aquadest), and dried again on filter paper in a laminar airflow cabinet. The sterilized wood samples were used to bait pathogens using the carrot baiting method and were stored for 7-10 days until fungal mycelium grew. The growing fungal mycelium was then isolated on malt extract agar (MEA) and stored at 25 °C (room temperature).

Morphological Characteristics

The isolates incubated for 10 days on MEA medium were then observed for their characteristics. Macroscopic observations of

fungal colonies included color, mycelial growth patterns, shape, and margins. Microscopic observations were conducted using an Olympus CX33 microscope with the assistance of Optilab, Advance Plus software, and Image Raster. These observations focused on the shape and size of perithecia, conidia, spores, ascomata, conidiospores, and chlamydospores. Each isolate was measured 100 times for repetition.

DNA Extraction, Amplification, Sequencing and Phylogenetic Analysis

Pure isolates were cultured in Potato Dextrose Broth (PDB) medium for 10 days at room temperature (25 °C). After 10 days, the mycelium was dried and ground using a mortar. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA). The concentration and purity of the DNA were measured using a NanoDrop ND-1000 spectrophotometer. Polymerase Chain Reaction (PCR) amplification and sequencing were performed using two β -tubulin primers, β T1a and β T1b (Pratama et al., 2021a), and internal transcribed spacer (ITS) primers, ITS1 and ITS4 (Pratama et al., 2021b). PCR was carried out using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The PCR mixture consisted of 12.5 μ L of MyTaq Red Mix 2x (Meridian Life Science Inc, USA), 0.5 μ L of each primer, 9.5 μ L of water, and 2 μ L of DNA template. The thermal cycling program included an initial denaturation phase at 95 °C for 1 minute, followed by 30 cycles of denaturation (95 °C for 15 seconds), annealing (55 °C for 15 seconds), and extension (72°C for 10 seconds). The amplification process concluded with a final extension step at 72 °C for 10 minutes, and the PCR products were stored at 10 °C. The PCR products were separated on a 1% agarose gel through electrophoresis at 95 volts for 25 minutes. 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 the GelDoc Go Imaging System (Bio-Rad). PCR products were sequenced by 1st Base Malaysia, and the resulting DNA sequences were compared with the GenBank database using BLAST nucleotide searches at the National Center for Biotechnology Information (NCBI). Relevant sequences were further processed and analyzed using BioEdit software (Pratama et al., 2023b).

The sequences of *Ceratocystis* species closely related to *Acacia mangium* were retrieved from GenBank. Phylogenetic sequences from various gene regions were aligned using Mesquite v3.5 and manually corrected. A phylogenetic tree, based on the combined ITS and β -tubulin gene dataset, was constructed and analyzed as a single dataset. Maximum Parsimony (MP) analysis was performed in MEGA v.11 with 1,000 bootstrap replicates to assess the relationships (Pratama et al., 2023b).

Koch's Postulates and Host Range Test

Koch's postulates were tested on *A. mangium*, and a host range test was conducted using seedlings of *Acacia crassicarpa* and *Artocarpus heterophyllus*. These plants had stem diameters of 2-3 cm and heights of less than 1 m. The pathogenic potential

of the isolates was evaluated using the bark-under inoculation method described by (Muslim *et al.*, 2022). The bark was wounded to expose the cambium using a 4 mm cork borer, and agar discs containing mycelium were taken from the edges of 2-week-old cultures grown on 2% MEA (Suwandi *et al.*, 2021). *Ceratocystis* isolates were then placed with the mycelium side facing the cambium. Ten plants from each tree species were inoculated, with sterile MEA plugs used as controls. All inoculation points were sealed with tape, and the ends of the tree stems were covered with polyethylene film to prevent desiccation of the inoculum and cambium while also reducing contamination.

Each plot row in the block contained ten replications per treatment. After 45 days, lesion length and percentage of plant death were recorded. Representative wood samples were collected from within the lesions, outside the inoculation area, and the pathogen was re-isolated and sequenced for Koch's postulate testing. The pathogenicity test data were analyzed using the SAS University Edition software package. Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test were used to identify significant differences among treatment means.

RESULTS

Symptoms of Fungal Infection on Diseased Trees

Acacia mangium affected by *Ceratocystis* disease is commonly found in plants aged 1-5 years, characterized by symptoms such as wilting leaves and a color change from green to yellow (Figure 1a). The plants gradually wilt until the leaves eventually dry out (Figure 1b). The plants begin to die completely 4-5 months after the wilting symptoms appear. On the sapwood of the tree, there are lesions in the form of scratch-like lines that are black and gray in color (Figure 1c). Lesions also extend into the vascular tissue, and fungal growth causes the vascular tissue to turn black (Figure 1d). The bark of the affected plants becomes sunken and cracked. Lesions are easily found in the

areas of bark that have been damaged by stem cankers, pruning wounds, insect holes such as ambrosia beetle infestations, or damage from animals such as broken branches caused by herbivores, or bites from monkeys and squirrels.

The intensity of the disease from 2022 to 2024 increased at all observation sites, reaching 100%. The disease incidence in 2022 ranged from 8.8% to 56.3%, with disease severity intensity ranging from 7.5% to 55.2%. In 2023, the disease incidence increased to 21.1% to 81.6%, with severity intensity ranging from 18.9% to 80.5%. By 2024, the disease incidence had become very severe, with an incidence range of 44.7% to 100% and severity ranging from 43.1% to 100%. The intensity and incidence reaching 100% indicate that the disease has caused the death of all affected plants (Table 1).

Morphological Characteristics

The morphology of *Ceratocystis* colony isolates grown on PDA medium showed different colors. Isolates CAW30653 and CAW30654 were very dark grayish-brown with moderate mycelial growth patterns, irregular shapes, and undulate margins. Isolate CAW30655 was brown with moderate mycelial growth, circular shape, and undulate margins. Isolate CAW30656 was dark brown with moderate mycelial growth, irregular shape, and undulate margins (Figure 2 & Table 2).

Based on the comparison of *Ceratocystis* isolate sizes, all isolates had globose perithecia, with the length of the perithecium base ranging from 131.20 to 280.21 and the width ranging from 121.42 to 373.46. The neck of the perithecium was straight, with a length ranging from 316.21 to 768.13. The ostiolar hyphae of all isolates were divergent, with lengths ranging from 22.12 to 46.17. The ascospores were cap-shaped, with widths ranging from 3.10 to 5.89 and lengths ranging from 9.12 to 33.17. The cylindrical conidia measured 10.12 to 33.17 in length and 2.11 to 7.71 in width, while the barrel-shaped conidia ranged from 8.12 to 14.12 in length and 4.12 to 8.52 in width. The chlamydospores of all isolates were globose to pyriform, with



Figure 1: Symptoms of *Ceratocystis* disease in *A. mangium* in the field. a) Leaf color changes and wilting, b) dry and dead plant leaves, c) lesions on sapwood and d) development of lesions on vascular tissue

lengths ranging from 8.14 to 18.03 and widths ranging from 4.28 to 18.82 (Table 3 & Figure 3).

Phylogenetic Tree

The PCR amplification results showed a fragment size of approximately 550 bp for the ITS and β -tubulin gene regions. The product sequences were then deposited in the GenBank database, where they were compared with other *Ceratocystis* species. BLAST searches using the ITS and β -tubulin genes in GenBank revealed that the *Ceratocystis fimbriata* isolate had a sequence that was 99% identical to *Ceratocystis fimbriata* found in *Lansium domesticum* plants. The evolutionary history was inferred using the Maximum Parsimony method. The

consistency index was 0.976744 (0.833333), the retention index was 0.913043 (0.913043), and the composite index was 0.891810 (0.760870) (Figure 4).

Koch's Postulates and Host Range Test

Koch's postulate test showed that all isolates were able to infect *Acacia mangium* plants, with lesion lengths ranging from 18.02 to 40.50 cm and a mortality rate of 60-100%. Host range testing using *Acacia carsicarpa* showed that all isolates were able to infect the plants, with lesion lengths ranging from 8.47 to 21.51 cm and a mortality rate of 10-50%. For *Artocarpus heterophyllus* plants, the isolates also resulted in lesion lengths ranging from 9.78 to 25.13 cm, with a mortality rate of 30-70%. Isolates CAW30653 and CAW30655 had strong pathogenicity, with average lesion lengths of 11.29-40.50 cm and mortality rates of 50-100%. Meanwhile, isolates CAW30654 and CAW30656 showed moderate pathogenicity, with average lesion lengths of 8.47-18.69 cm and mortality rates of 10-70% (Table 4).

Table 1: Incidence and intensity of Ceratocystis wilt disease in acacia in Banyuasin

Location (number of plants)	Disease Intensity and Incidence (%)					
	May 2022		July 2023		October 2024	
	IP	IS	IP	IS	IP	IS
Pangkalan Balai (n=47)	13.3	14.9	23.9	25.5	43.1	44.7
Sungai Pinang (n=77)	40.6	42.9	65.6	67.5	92.2	93.5
Rambutan (n=87)	55.2	56.3	80.5	81.6	100	100
Sukajadi (n=57)	7.5	8.8	18.9	21.1	43.9	45.6

IP=Disease Intensity; IS=disease incidence

DISCUSSION

Based on surveys conducted from 2022 to 2024, *Ceratocystis* wilt disease has been newly reported to attack and spread

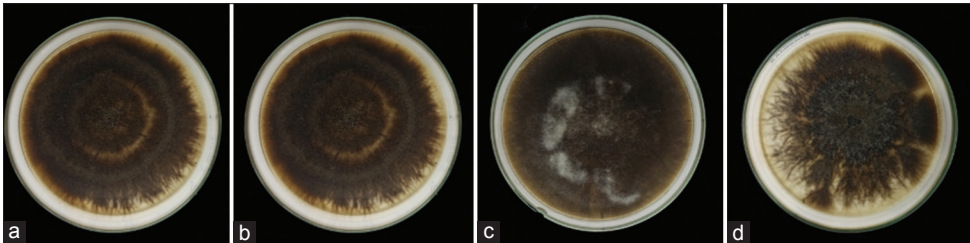


Figure 2: Morphology of *Ceratocystis* isolate colonies on PDA medium 14 days incubation. a) CAW30653, b) CAW30654, c) CAW30655 and d) CAW30656



Figure 3: Morphological characteristics of *Ceratocystis* isolated from *Acacia mangium* stem lesions: a) The base of the perithecium is globose with a long neck, b) divergent ostiolar hyphae, c) barrel-shaped conidia, d) chlamydospores, e) cap-shaped ascospores, f) cylindrical conidia, and g) phialid. Scale bar: a=100 μ m; b, c, d, e=10 μ m; f=5 μ m

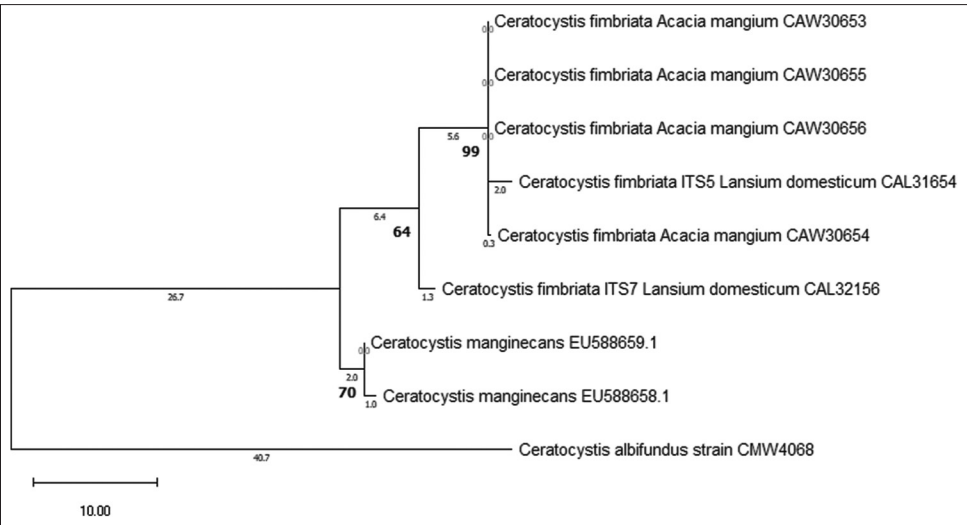


Figure 4: Phylogenetic relationships of *Ceratocystis* isolates from a dataset of ITS and β -tubulin regions sequences alignment. The phylogram was obtained using the heuristic search option based on parsimony with *Ceratocystis albifundus* as the out-group

Table 2: Characteristics of *Ceratocystis* isolate colonies on PDA medium

Isolate Code	Potatoes Dextrose Agar (PDA)				
	Colony Color				
	Color	Code	Mycelium Growth Pattern	Form	Margin
CAW30653	Very dark grayish brown	10YR; 3/2	Moderat	Irregular	Undulate
CAW30654	Very dark grayish brown	10YR; 3/2	Moderat	Irregular	Undulate
CAW30655	Brown	10YR; 4/3	Moderat	Circular	Undulate
CAW30656	Dark brown	10YR; 3/3	Moderat	Irregular	Undulate

Table 3: Comparison of morphological sizes of *Ceratocystis* isolates from *Acacia mangium*

Morphological Characteristics of Isolates	CAW30653	CAW30654	CAW30655	CAW30656
Perithecium				
Form	Globose	Globose	Globose	Globose
Base of the perithecium (p)	136.21-250.43	137.40-260.31	141.23-280.21	131.20-247.32
Base of the perithecium (l)	128.23-373.38	127.23-213.13	125.21-217.22	121.42-245.12
Perithecium Neck	Straight	Straight	Straight	Straight
Neck (p)	321.23-768.13	341.16-711.62	321.14-713.11	316.21-721.52
Ostiolar hyphae				
Form	Divergent	Divergent	Divergent	Divergent
Ostiolar hyphae (p)	23.11-45.13	22.12-44.12	23.15-46.17	22.19-42.85
Ascospores				
Hat-shaped ascospores (p)	3.24-5.19	3.10-5.89	3.24-5.73	3.53-5.80
Hat-shaped ascospores (l)	2.62-3.19	2.12-3.54	2.23-3.89	2.13-3.19
Cylindrical conidia (p)	10.12-25.19	9.85-29.14	9.56-27.14	9.12-33.17
Cylindrical conidia (l)	2.11-6.27	2.33-6.51	2.24-6.31	2.15-7.71
Barrel-shaped conidia (p)	8.12-14.12	8.14-12.56	8.35-11.15	8.23-12.68
Barrel-shaped conidia (l)	4.99-8.12	4.21-6.14	4.23-8.41	4.12-8.52
Chlamydospores				
Form	Globose to pyriform	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (p)	8.14-17.25	9.27-17.26	8.52-17.16	8.14-18.03
Chlamydospores (l)	5.23-15.11	5.78-18.82	4.97-16.92	4.2815.72

p=length; l=width; Measurements are presented in minimum x maximum format; All measurements are in μ m

widely in the Banyuasin area, originating from Rambutan. Currently, this wilt disease has been found affecting *Acacia mangium* plants in other locations such as Pangkalan Balai, Sungai Pinang, and Sukajadi. The initial symptoms are marked by cracked and broken tree bark, and some trees show symptoms of stem canker. On the sapwood, brown

lesions form, which gradually turn black and elongate into scratch-like patterns. The leaves of the plants turn yellow, wilt, dry out, and eventually the plants die. These symptoms are similar to those reported by (Tarigan *et al.*, 2011) and (Syazwan *et al.*, 2021), which affected *Acacia mangium* plants in Riau and Malaysia.

Table 4: Results of Koch's postulates and host range tests

Isolates	Test Host	<i>Acacia mangium</i>		<i>Acacia carsicarpa</i>		<i>Artocarpus heterophyllus</i>	
		PL (cm)	TM	PL (cm)	TM	PL (cm)	TM
CAW30653	10	40.13 ^c	10/10	19.11 ^c	5/10	25.13 ^d	7/10
CAW30654	10	18.02 ^b	6/10	9.78 ^b	2/10	9.92 ^b	3/10
CAW30655	10	40.50 ^c	10/10	21.51 ^d	5/10	11.29 ^c	5/10
CAW30656	10	18.69 ^b	7/10	8.47 ^b	1/10	9.78 ^b	3/10
Control (MEA)	10	0.2 ^a	0/10	0.2 ^a	0/10	0.2 ^a	0/10

PL: Lesion Length; TM: Dead Plant

Wounds on trees and the formation of cankers allow pathogens to enter and infect *Acacia mangium* plants. Many trees show wounds caused by pruning, squirrel bites, cracks from wind, and insect holes. The spread of this wilt disease can be caused by natural wounds from wind or attacks by other animals (Mortenson *et al.*, 2016). *Xyleborus affinis*, *Xylosandrus crassiusculus*, and *Hypothenemus* sp. are vectors in the spread of wilt disease on *A. mangium* in Malaysia (Syazwan *et al.*, 2021). The pathogen responsible for wilt on *Acacia mangium* is soilborne (Barnes *et al.*, 2023). Plant resistance is influenced by several factors, one of which is systematic resistance (Ali *et al.*, 2018). Horizontal gene transfer can occur through host enzymes targeting pathogen cells or dying host tissues, allowing hyphae to enter (Ghaly *et al.*, 2024). The geographic location of each site, namely Rambutan, Pangkalan Balai, Sungai Pinang, and Sukajadi, may be a factor that increases the severity of a disease (Zulaika *et al.*, 2018).

Based on the results of morphological identification, the isolate was classified into the genus *Ceratocystis*, possessing a globose perithecium that is black in color, and having ascospores and chlamydospores. The shape, color, and size of the isolate obtained were not significantly different from those of *Ceratocystis* isolates that infect *A. mangium* in Malaysia (Syazwan *et al.*, 2021). The identity of *C. fimbriata* as a pathogen associated with wilt disease on *A. mangium* was determined based on the comparison of ITS and β -tubulin DNA sequences, which showed a 99% similarity to *C. fimbriata* infecting *Lansium domesticum*, with the same symptoms, including progressive crown loss leading to tree death, and the bark around the lesions and wood turning dark blue to brown on the affected stem. In general, these symptoms are similar to those of *C. fimbriata* infecting jackfruit (Pratama *et al.*, 2021b) and causing sudden decline in bullet wood disease (Pratama *et al.*, 2021a), making it a potential cause of damage and destruction to *A. mangium* in Indonesia.

Koch's postulate test showed that this pathogen is the main cause of wilt and sudden death in *Acacia mangium* in Banyuasin. Pathogenicity tests on *A. mangium* indicated that the incidence and intensity of the attack were very high, reaching 100%, leading to wilting and plant death. Host range tests on *A. carsicarpa* and *A. heterophyllus* showed that the isolate could infect both with a mortality rate of 50 to 70%. *Ceratocystis fimbriata* is known as a pathogen with a very broad host range, including Meliaceae, represented by *Lansium domesticum* (Suwandi *et al.*, 2021), Moraceae, represented by *Artocarpus heterophyllus* (Pratama *et al.*, 2021b), and Myrtaceae, represented by *Eucalyptus*

(Kamgan Nkuekam *et al.*, 2012). This supports the perspective that *C. fimbriata* has a wide host range, making it potentially capable of infecting other trees not previously mentioned.

CONCLUSIONS

The sudden wilt disease in *A. mangium* caused by *C. fimbriata* has spread widely to plantations in various areas of Banyuasin district. In addition, the population consists of individuals with uniform morphological characteristics and genetic diversity, and it is highly pathogenic to *A. mangium*. *Ceratocystis fimbriata* is also pathogenic to all plants used in the host range tests, making it a serious threat to Indonesia's biodiversity.

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