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First report of *Ceratocystis fimbriata* causing wilt disease of soursop in South Sumatra, Indonesia

RAHMAT PRATAMA, AHMAD MUSLIM♥, SUWANDI SUWANDI, SUPARMAN SHK
Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Jl. Raya Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia. Tel.: +62-711-580739, ♥email: a_muslim@unsri.ac.id

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Abstract. *Pratama R, Muslim A, Suwandi S, Suparman Shk. 2023. First report of* Ceratocystis fimbriata *causing wilt disease of soursop in South Sumatra, Indonesia. Biodiversitas 24: 6711-6721. Ceratocystis* fungus is a well-known pathogen that frequently attacks agroforestry plants. Symptoms of canker and wilt in soursop plants (*Anona muricata* L.) similar to those of ceratocystis disease with 57-100% mortality were first observed in South Sumatra, Indonesia in 2021. This study aimed to describe the symptomatology of the new disease and characterize isolates of *Ceratocystis fimbriata* Ellis & Halst. obtained from diseased soursop plants. The identification of this pathogen involved a comprehensive polyphasic analysis, which combined morphological and molecular characteristics derived from specific genomic regions (ITS and β-tubulin). Morphological and phylogenetic analysis revealed that all pathogen isolates were identified as *C. fimbriata*. All the population showed uniform morphology, comprising ITS5 and ITS7b haplotypes. The pathogenicity test indicated that *C. fimbriata* induced similar symptoms similar to natural symptoms, resulting in mortality rates of 50-90%. The results of pathogenicity tests of *A. muricata* isolates showed their high pathogenicity toward other *Ceratocystis* hosts plants, including *Artocarpus heterophyllus* Lam. and *Acacia mangium* Willd. These tests resulted in the death rates of 15-50% and 75-100%, respectively, which closely resembled the pathogenicity observed on *A. muricata.* Both isolates of the ITS5 haplotype exhibited nearly identical pathogenicity on the three hosts, while ITS7b displayed higher aggressiveness. This is the the first report of *C. fimbriata* as a causal agent of wilt in soursop trees in Indonesia and worldwide, highlighting the importance of understanding this pathogen and its management to mitigate its impact on agroforestry plants.

Keywords: *Anona muricata, Ceratocystis fimbriata*, Ceratocystidaceae, South Sumatera, wilt disease

Abbreviations: Bt: Beta tubulin; ITS: Internal Transcribed Spacer

INTRODUCTION

Graviola, commonly known as soursop (*Anona muricata* L.), is a native plant of America that holds significant importance in Indonesia, where it is widely cultivated in home gardens, community orchards, and on the edges of forests. The popularity of soursop fruit among people is attributed to its delicious taste, leading to its versatile use in various preparations such as syrup, candies, beverages, ice cream, and shakes. Beyond its pleasant taste, this native plant boasts numerous health benefits and therapeutic effects, including anti-cancer, anticonvulsant, anti-rheumatic, antiparasitic, antimalarial, hepatoprotective, and antidiabetic properties (Moghadamtousi et al. 2015; Coria-Tellez et al. 2018). *A. muricata* is rich in vitamins and minerals that have the potential to bolster the immune system, helping to protect the body from infections and illnesses (Illango et al. 2022). Moreover, soursop leaves have a rich history of use in traditional medicine, with their extract being particularly abundant in bioactive compounds. These compounds hold significant potential for utilization as therapeutic drugs or nutraceuticals (Rady et al. 2018; Mutakin et al. 2022).

In the garden of Universitas Sriwijaya, numerous soursop plants have been observed to exhibit symptoms of wilting leaves and the presence of longitudinal streaks resembling canker on the stem, ultimately leading to their death. Surprisingly, there have been no reports of this disease affecting soursop plants in Indonesia or worldwide. The symptoms observed are very similar to those of *Ceratocystis fimbriata* Ellis & Halst. wilt, which has been reported in various studies to be a fatal disease affecting forest and agroforestry plants. *C. fimbriata* belongs to Ascomycete (Microascales, Ceratocystidaceae), an indigenous soil-dwelling pathogen (Ferreira et al. 2017). It can easily spread to new locations through contaminated tools and infected propagative materials (Chi et al. 2019; Chi et al. 2021), leading to a devastating wilt of a diverse array of economically valuable plants. Significant examples of its hosts include wilt disease on *Acacia mangium* Willd. (Syazwan et al. 2021), wilt of *Eucalyptus grandis* W.Hill ex Maiden × *Eucalyptus urophylla* S.T.Blake (Roux et al. 2020), *Mangifera indica* (Al Adawi et al. 2013a; Pratama et al. 2023), jackfruit (*Artocarpus heterophyllus* Lam.) (Pratama et al. 2021a), bullet wood (*Mimosops elengi* L.) (Pratama et al. 2021b). *Ceratocystis* causing lethal wilt disease, has also spread widely across various districts as centers of South Sumatra duku (*Lansium domesticum* Corrêa) plantations as in Ogan Komering Ilir, Muara Enim, Musi Rawas, North Musi Rawas, Ogan Komering Ulu, and Musi Banyuasin with varying levels of incidences (Suwandi et al. 2021; Muslim et al. 2022). As this disease has the potential to disrupt plant production (Nasution et al. 2019),

its impact on soursop plants could have significant economic implications.

Plants that are infected with *Ceratocystis* typically show gradual drooping of their leaves, branches, or even the entire plant. This withering can occur rapidly and is often a prominent sign of disease. *Ceratocystis* comprises a group of pathogenic fungi that target the xylem of plants, initiating hyphal growth and spore production within the vessels. These vessels, in turn, obstruct tracheal elements and induces water stress to plants. When infected by this pathogen, plants first loss their dark green leaf color or leaf discoloration, followed by wilting, drying, and eventual death, with dry leaves persisting on the plant. A distinctive feature of e infection is the observation of brown radial lines upon cutting the infected stem of the plant. The plant infected by *Ceratocystis* commonly produces gum or types of resin in response to fungal infection (Ferreira et al. 2017; Xu et al. 2022). Considering the significance of the impact of this disease on forest and agroforestry plants, particularly indigenous Indonesian plants. This study aimed to observe the distribution of wilt disease on *A. muricata* across various districts in South Sumatra. Additionally, the investigation examined the characteristics of pathogenic isolates and conducted pathogenicity tests on other economically valuable plants.

MATERIALS AND METHODS

Sample collection and fungal isolation

This study was conducted over a period spanning from January 2021 to December 2022, in ten regencies within South Sumatra Province. Samples were collected from diseased soursop plants showing wilting symptoms and discoloration in the vascular tissue, indicative of *Ceratocystis* infection. The collection of samples involved making an incision on the bark and obtaining tangential longitudinal sections (approximately 50 mm) from the newly infected xylem, where the fungus was present. The selected soursop plants for sampling ranged in age from 1 to 3 years, as these age groups were found to be significantly impacted in plantations.

Samples of diseased wood underwent carrot baiting where discolored wood was placed between two slices of carrot (Oliveira et al. 2021). The first slice of carrot was treated with streptomycin sulfate (100 mg/L) and incubated at room temperature to stimulate fungal sporulation on the carrot slices. Additionally, wood pieces were sterilized with sodium hypochlorite (NaClO) for 5 minutes, rinsed with distilled water, and dried in a laminar airflow. These wood pieces were directly planted on Malt Extract Agar (MEA) medium at room temperature (25°C) for 7-10 days to induce sporulation directly on the MEA. The ascospores developing as single masses at the apex of ascomata on wood slices planted directly on MEA media or infected carrots were transferred to new Petri dishes containing 2% MEA (MEA, 20 g/L malt, 20 g/L agar) (Biolab, Midrand, South Africa). The cultures were then incubated at 25°C for 8-10 d.

Morphological characterization

Three isolates, SIC1, SIC2, and SIC3, derived from *A. muricata* trees, were subjected to a comparative analysis of their characteristics. For morphological characterization, *Ceratocystis* isolates were cultivated on 2% MEA media and incubated at 25°C for 10 days. Fungal cultures were placed on glass slides and a drop of lactophenol was added and observed under a light microscope. For each isolate, measurements were recorded 100 times for ascomata, hat-shaped ascospores, bacilliform conidia, barrel conidia, and chlamydospores.

DNA extraction, amplification, sequencing, and phylogenetic analysis

Pure cultures of *Ceratocystis* isolates were cultured in potato dextrose broth (PDB) at 25°C for 10 days, after which DNA extraction was carried out. The mycelium obtained from the PDB culture was separated, dried, and subsequently ground into a fine powder using a mortar and pestle. DNA extraction was performed using the Zymo Research ZR Fungal/Bacterial DNA MiniPrep™ kit (Irvine California, USA). To assess the concentration and purity of extracted DNA, measurements were conducted using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, USA).

PCR amplification and sequencing of two gene regions, βT1a (TTCCCCCGTCTCCACTTCT TCATG) and βT1b (GACGAGATCGTTCATGTTGAACTC) (Oliveira et al. 2015a), as well as the ITS, ITS1 (5k TCC GTA GGT GAA CCT GCG G 3k) and ITS4 (5k TCC TCC GCT TAT TGA TAT GC 3k) (Kanzi et al. 2020), were performed in this study. Amplification was carried out in a 50 µL reaction containing 20 µL Master Mix (Eppendorf, Germany) (25 mM $MgCl_2$, 0.06 U/µL Taq-DNA Polymerase, 0.2 mM of each dNTP), 1 µL of each forward and reverse primer, 1 µL DNA template, and 27 µL sterile water. The PCR was performed using the C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycle consisted of an initial denaturation at 94°C for 3 minutes, followed by 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds, and 72 °C for 1 minute. A final extension step was performed at 72°C for 10 minutes, and subsequent storage of the PCR products occurred at 10°C. Furthermore, the PCR amplicons were sent to 1st BASE (Malaysia) for sequencing. The obtained DNA sequences were compared with the GenBank database using a BLAST search, which was conducted at the National Center for Biotechnology Information (NCBI), Bethesda, USA. Relevant sequences were then transferred and processed using BioEdit software (Firmino et al. 2016). Phylogenetic trees were visualized and edited in MEGA v.11, utilizing maximum parsimony (MP) analysis with 1000 bootstrap replicates (Kumar et al. 2016). For the construction of the phylogenetic trees, *C. virescens* was employed as the outgroup taxon, while the in-group was considered monophyletic. The ITS haplotypes were designated by numbers according to the numerical system of Harrington et al. (2014).

Inoculation trials

Anona muricata L. seedlings were used for pathogenicity test (Koch postulates). The pathogenicity test of *Ceratocystis* isolates, including SIC1, SIC2, and SIC3 (Table 1), were evaluted using *A. heterophyllus* and *A. mangium* seedlings. For the experiment, 5-month-old seedlings with stem diameters ranging from 2 to 3 cm and heights of 80-100 cm were sourced from local suppliers. These seedlings were potted in 20;19;14 cm (top diameter; high; lower diameter) pots filled with peat soil + manure. To initiate the inoculation process, plants were carefully wounded using a sterile surgical blade, creating 10 mm long incisions at a height of 20 cm from the ground surface. Subsequently, mycelium agar (4 mm in diameter) was inserted into each wound site. Each *Ceratocystis* isolate was used to inoculate twenty per host plant, while an equivalent number of seedlings were inoculated with sterile MEA as control. The experimental design was conducted in a completely randomized design. To protect the inoculated wounds, the plants were covered with moist sterile cotton and sealed using parafilm.

The inoculated plants were placed in an experimental house and watered twice a day. Daily observations were conducted to monitor the development of symptoms and mortality. After 60 days, the bark tissue from the inoculated seedlings was incised both above and below the inoculation site, and the length of lesions was measured. To re-isolate the inoculated pathogen, wood samples were collected from the lesion edges and grown on MEA plates. Alternatively, the majority of the samples were placed between two carrot slices to confirm adherence to the postulates of Koch. For data analysis, the lesion length data among isolates and hosts were subjected to statistical examination using the SAS University Edition software package. Analysis of variance (ANOVA) was conducted, and Tukey's honestly significant difference (Tukey's HSD) test was used to determine when there were any significant differences in the average comparisons of different treatments.

RESULTS AND DISCUSSION

Sample collection and disease symptoms

The symptoms of wilting and sudden death in soursop plants were observed for the first time in Indonesia and globally. During sample collection it was observed that soursop plants suffering from infection exhibited wilting of leaves and subsequent drying up (Figure 1A). Upon incision of the sapwood, discolorations were evident, manifested as patterns resembling scratches and black discoloration (Figure 1B). The development of disease in the sapwood caused notable changes in the leaves, which became yellow, followed by browning and wilting (Figure 1C). Additionally, disease spread to the heartwood of soursop plant, disrupting the vascular tissues, which ultimately led to wilting and eventual plant die (Figure 1D). On the stem, characteristic features such as black-colored patches, gummosis, and stem cracking were also evident on the outer bark.

During the survey it was observed that there was significant mortality of soursop plants in two out of ten regencies in South Sumatra (Table 1). In Gelumbang, 100% soursop plant (5 out of 5 plants) dies due to the disease, while in soursop plantations in Indralaya, the mortality rate was 57% (29 out of 51 plants). The disease was not found in other areas where soursop plants were abundant. A total of 10 isolates were successfully isolated, of which there were three isolates of *Ceratocystis*, namely SIC1 and SIC2 from Ogan Ilir, SIC3 from MuaraEnim. The isolate cultures obtained were preserved in the Culture Collection (CMW), Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya.

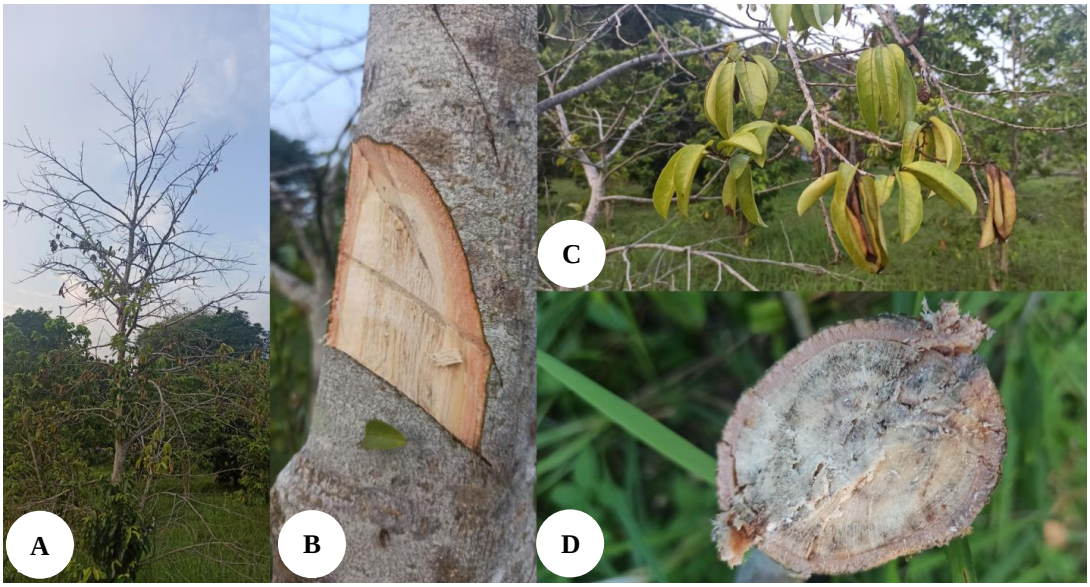

Figure 1. Symptoms of *Ceratocystis* wilt disease on soursop plants. A. Soursop plant affected by wilting disease. B. Discoloration in sapwood. C. Wilting and yellowing leaves in the diseased plant. D. Fungal development in soursop tree heartwood

Morphological characterization, DNA extraction, amplification, sequencing, and phylogenetic analysis

Macroscopic observations of the three isolates showed distinct colony shapes (Figures 2A-C). SIC1 and SIC3 exhibited filamentous appearance at the edges, while SIC2 was irregular in shape. Despite the different colony shapes, all isolates showed the same dark brown to black colony color.

The structural dimensions of ascomata, ascospores, and chlamydospores had no significant differences among the studied isolates (Table 2). Ascomatal bases were dark brown to black, with a subglobose to globose shape, and measured (n = 100) 145.7-345.2 × 105.2-249.2 µm (Figure 3A). The ascomata necks were erect, occasionally curved, exhibited a black color at the base. Toward the apex, ascomata necks became subhyaline, with a smooth to crenulate surface. The measured length, including ostiolar hyphae, ranged from 315.6 to 781.2 µm (Figure 3B). Phialides appeared pale brown to hyaline (Figure 3C), while chlamydospores were oval, characterized by a thick-walled and smooth structure, measuring 6.7-14.9 × 5.9-11.5 µm (Figures 3D-E). Ascospores exhibited a hat-shaped form, measuring 3.1-5.2 × 1.9-5.6 µm (Figure 3F). Secondary conidia hyaline, aseptate, barrel-shaped conidia measured 6.1-11.2 × 3.9-7.2 µm (Figure 3G), while primary conidia were hyaline, aseptate, cylindrical or bacilliform conidia measured 10.1-26.9 × 3.1-7.1 µm (Figure 3H).

Table 1. Incidence of *Ceratocystis* wilt on *Annona muricata* of South Sumatra

Locations (city/district)	Plants observed	Diseased plants	Disease incidence (%)
Gelumbang (MuaraEnim)	5	5	100
Indralaya (Ogan Ilir)	51	29	57
Sekayu (Musi Banyuasin)	22	0	0
Kayuagung (Ogan Komering Ilir)	17	0	0
Lembak (Prabumulih)	7	0	0
Pangkalan Balai (Banyuasin)	11	0	0
Jakabaring (Palembang)	3	0	0
Batan Pelita (Ogan Komering Ulu Timur)	14	0	0
Rupit (Musi Rawas Utara)	13	0	0
Baturaja (Ogan Komering Ulu)	9	0	0

Table 2. Morphology of selected *Ceratocystis fimbriata* isolates from different districts in South Sumatra

Isolates/morphological characters [a]	Ogan Ilir		MuaraEnim
	SIC1	SIC2	SIC3
Ascomatal bases			
Shape	Globose	Globose	Globose
Ascomatal base (w)	163.2 to 345.2	158.9-292.5	145.7 to 286.4
Ascomatal base (l)	105.2 to 249.2	112.1 to 216.5	106.3 to 210.3
Ascomatal necks	Straight	Straight	Straight
Neck (l)	402.1 to 759.3	361.6 to 781.2	315.6 to 718.3
Neck (w) top	8.6 to 15.3	7.12 to 17.4	8.3 to 19.3
Neck (w) bottom	23.2 to 45.6	21.4 to 51.6	22.4 to 46.2
Ostiolar hyphae			
Shape	Divergent	Divergent	Divergent
Ostiolar hyphae (l)	30.3 to 41.7	30.1 to 40.5	31.3 to 41.6
Ascospores			
Hat-shaped ascospores (l)	3.1 to 5.2	3.1 to 5.2	3.2 to 5.1
Ascospores (w) without sheath	3.3 to 4.9	3.5 to 6.1	3.1 to 5.2
Ascospores (w) with sheath	1.9 to 5.3	2.2 to 5.1	2.5 to 5.6
Primary conidia (l)	10.8 to 26.9	10.1 to 17.8	11.2 to 26.1
Primary conidia (w)	3.3 to 7.1	3.1 to 5.8	3.1 to 5.5
Secondary Conidia (l)	6.1 to 10.2	6.3 to 10.8	6.2 to 11.2
Secondary Conidia (w)	3.9 to 5.9	4.1 to 7.2	4.2 to 7.1
Chlamydospores			
Shape	Globose to pyriform	Globose to pyriform	Globose to pyriform
Chlamydospores (l)	6.7 to 12.1	7.81 to 14.9	8.3 to 13.1
Chlamydospores (w)	5.9 to 8.9	6.2 to 11.4	6.4 to 11.5
Culture growth rate at [b] 10°C		0	0
15°C	3.1 to 3.3	2.1 to 2.3	3.0 to 3.5
20°C	3.4 to 3.9	3.1 to 3.6	3.6 to 3.8
25°C	5.2 to 5.5	4.8 to 5.2	4.4 to 5.0
30°C	3.0 to 3.7	3.4 to 4.0	3.2 to 3.9

Notes: [a] All morphological characters represent a minimum-maximum for 100 measurements for each morphological structure measured in µm; [b] Growth rate measurements represent an average of diameters of cultures measured in cm at each temperature after fourteen days

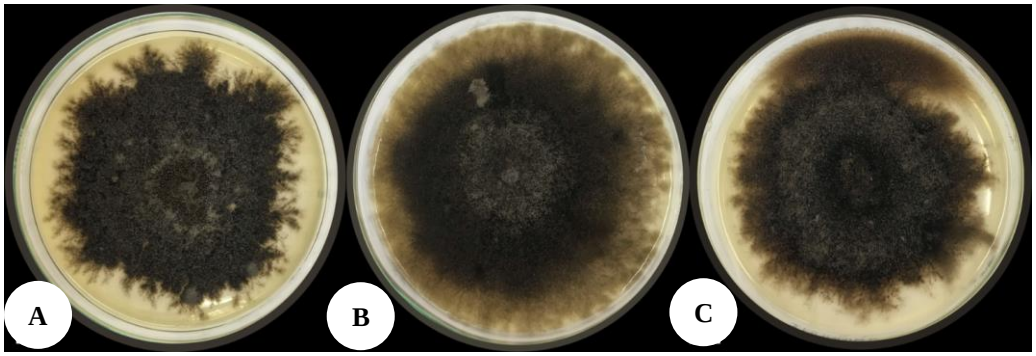

Figure 2. Isolates of *C. fimbriata* grown on malt extract agar (MEA) for 7 d at 25 C. A. SIC1; B. SIC2; and C. SIC3

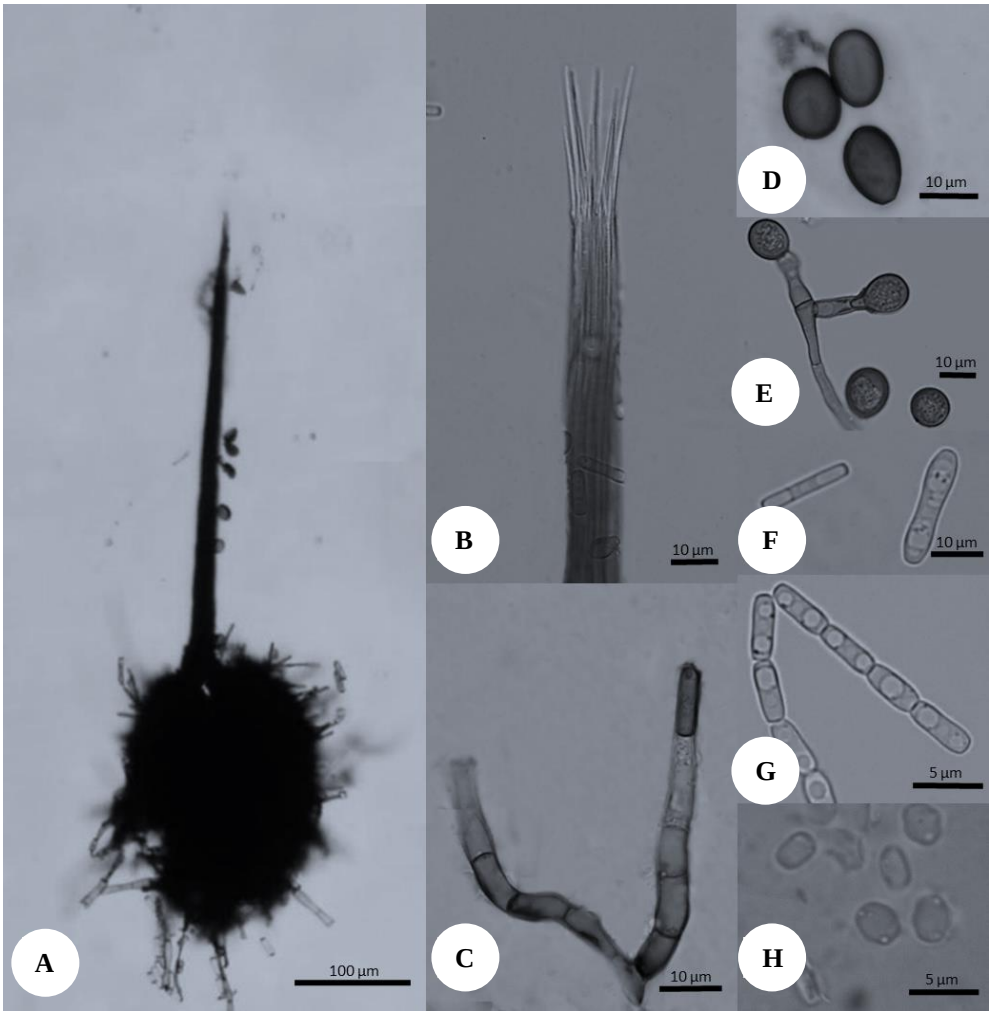

Figure 3. Morphology of *Ceratocystis fimbriata*. A. Perithecial-shaped ascomata. B. Divergent ostiolar hyphae. C. Phialide/conidiophore. D. Chain-shaped chlamydospores. E. Chlamydospores of various shapes. F. Barrel conidia. G. Cylindrical conidia. H. Ascospores

PCR amplification of the ITS and β-tubulin gene regions yielded fragment sizes of approximately 550 base pairs. The resulting sequence data were submitted to GenBank for comparison with other *Ceratocystis* isolates (Table 3). A BLAST search using the β-tubulin gene in GenBank demonstrated that isolates from *C. fimbriata* sensu stricto were closely grouped with sequences showing 99% identity (Figure 4). Based on the ITS gene data, isolates were predominantly characterized by ITS5 and a novel ITS haplotype referred to as ITS7b in *C. fimbriata* (Figure 5). The ITS haplotypes of *C. fimbriata* were designated by numbers. *C. variospora* served as the outgroup taxon in this analysis.

Table 3. Phylogenetic analysis of *Ceratocystis* isolates

Spesies	Haplotypes	Isolates number	Hosts	Origin	GenBank accession no.	
					ITS	β-tubulin
C. fimbriata	ITS1a	C1418	*Ipomoea batatas*	USA	AY157956	-
	ITS1	C1857	*Ficus carica*	Brazil	HQ157542	-
	ITS1b	CMW4797	*Eucalyptus* sp.	Congo	FJ236733	-
	ITSb	CMW9998	*Eucalyptus* sp.	South Africa	FJ236721	-
	ITS2	C1655	*Mangifera indica*	Brazil	HQ157546	-
	ITS3	C1440	*Eucalyptus* sp.	Brazil	HQ157544	-
	ITS3	CMW5328	*E. grandis*	Uganda	AF395686	-
	ITS4	C1442	*Eucalyptus* sp.	Brazil	HQ157545	-
	ITS5	**SIC1**	***Anonna muricata***	**Indonesia**	**Submitted**	**Submitted**
	ITS5	**SIC2**	***A. muricata***	**Indonesia**	**Submitted**	**Submitted**
	ITS5	CMW38737	*E. grandis*	Zimbabwe	KF878326	KF878335
	ITS5	C1345	*Eucalyptus* sp.	Brazil	AY157966	-
	ITS5	A59662	*Camellia sinensis*	China	KF650948	-
	ITS5	YM061	*Colocasia esculenta*	China	AM712445	-
	ITS5	P20053	*Punica granatum*	China	AM292204	-
	ITS5	C1	*Acacia* sp.	Vietnam	MF033455	MF040712
	ITS5	CMW22563	*A. mangium*	Indonesia	EU588656	EU588636
	ITS5	WRC	*Lansium domesticum*	Indonesia	MT229127	MW013766
	ITS6	C2055	*Mangifera* sp.	Brazil	HQ157548	-
	ITS6z	CMW13582	*Hypocryphalus mangifera*	Oman	KC261853	-
	ITS6z	WBC	*L. domesticum*	Indonesia	MT229128	MW013767
	ITS7b	CMW13851	*M. indica*	Oman	AY953383	EF433308
	ITS7b	**SIC3**	***A. muricata***	**Indonesia**	**Submitted**	**Submitted**
	ITS7b	CMW23634	*M. indica*	Pakistan	EF433302	EF433311
	ITS7b	CMW22579	*A. mangium*	Indonesia	EU588658	-
	ITS8a	CMW8856	*Citrus* sp.	Colombia	AY233867	-
	ITS8c	CMW17808	*Eucalyptus* sp	Colombia	EF127990	-
	ITS8e	CMW22092	*E. deglupta*	Ecuador	FJ151432	-
	ITS9	C1558	*M. indica*	Brazil	AY157965	-
	ITS9	C1914	*C. esculenta*	Brazil	HQ157540	-
	ITS10	C994	*M. indica*	Brazil	AY157964	-
	ITS10a	Cf4	*M. indica*	Brazil	EF042605	-
	ITS11	C1865	*C. esculenta*	Brazil	AY526286	-
	ITS12	C1926	*C. esculenta*	Brazil	HQ157541	-
	ITS14	C1688	*M. indica*	Brazil	AY526291	-
	ITS15	C925	*Gmelina arborea*	Brazil	AY157967	-
	ITS16	C924	*G. arborea*	Brazil	HQ157539	-
C. pirilliformis	Asian clade (AC)	CMW6569	*E. nitens*	Australia	-	DQ371652
	AC	CMW6579	*E. nitens*	Australia	-	DQ371653
C. polychroma	AC	CMW11424	*Syzygium aromaticum*	Indonesia	-	AY528966
	AC	CMW11436	*S. aromaticum*	Indonesia	-	AY528967
C. atrox	AC	CMW19383	*E. grandis*	Australia	-	EF070430
	AC	CMW19385	*E. grandis*	Australia	-	EF070431
C. neglecta	Latin American clade (LAC)	CMW17808	*E. grandis*	Colombia	-	EU881898
	LAC	CMW18194	*E. grandis*	Colombia	-	EU881899
C. colombiana	LAC	CMW5751	*Coffea arabica*	Colombia	-	AY177225
	LAC	CMW5761	*C. arabica*	Colombia	-	AY177224
C. cacaofunesta	LAC	CMW14803	*Theobroma cacao*	Ecuador	-	KJ631108
	LAC	CMW15051	*T. cacao*	Costa Rica	-	KJ601510
C. papillate	LAC	CMW8850	*Citrus × Tangelo hybrid*	Colombia	-	AY233875
	LAC	CMW8856	*Citrus limon*	Colombia	-	AY233874
C. fimbriata	LAC	CMW14797	*M. indica*	Brazil	-	EF433307
	LAC	CMW28907	*M. indica*	Brazil	-	FJ200270
	LAC	CMW1547	*I. batatas*	Papua New Guinea	-	EF070443
	LAC	C1421	*I. batatas*	USA	-	KF302689
C. fimbriatomima	LAC	CMW24174	*Eucalyptus hybrid*	Venezuela	-	EF190951
	LAC	CMW24176	*Eucalyptus hybrid*	Venezuela	-	EF190952
C. fimbriata	LAC	CMW21127	*A. crassicarpa*	Indonesia	-	EU588643
	LAC	CMW24664	*Eucalyptus hybrid*	China	-	JQ862720
	LAC	CBS115173	*Gmelina arborea*	Brazil	-	KF302700
	LAC	CBS14653	*Caffea arabica*	Suriname	-	KF302702
C. platani	LAC	CMW14802	*Platanus occidentalis*	USA	-	EF070425
	LAC	CMW23450	*P. occidentalis*	Greece	-	KJ601513

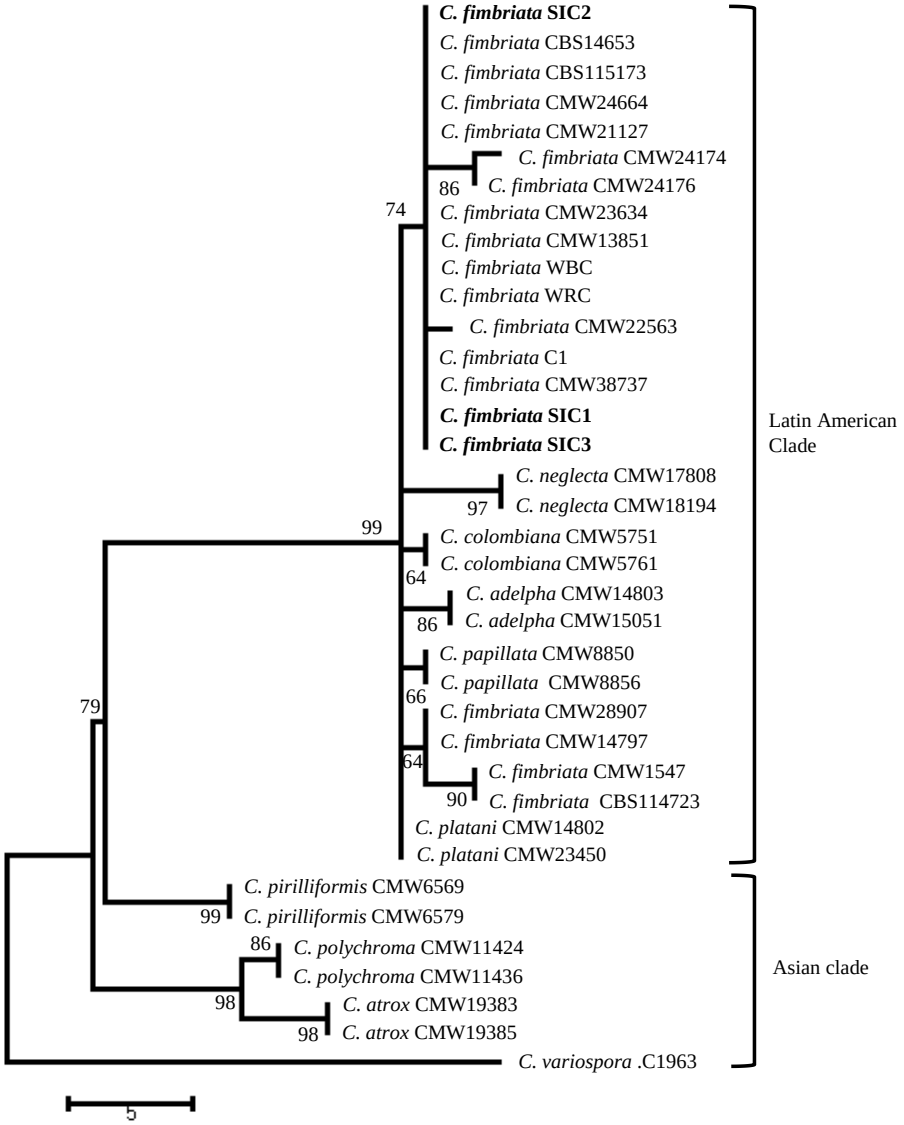

Figure 4. The phylogenetic trees resulting from the maximum parsimony analysis of the β-tubulin sequence, which showed the relationship between *Ceratocystis fimbriata* from the *Annona muricata* trees in Indonesia (highlighted in bold) and other species within the Latin American and Asian clade of the *C. fimbriata* species complex. As an outgroup, *C. variospora* was used in the analysis

Pathogenicity test (Koch Postulates)

The pathogenicity test result exhibited that *A. muricata* plants showed similar symptoms observed in the field, including wilting leaves, yellowing (Figure 6A), and lesion formation in sapwood (Figure 6B) and vascular tissue (Figure 6C), with a mortality rate ranging from 50 to 90%. The successful reisolation of *C. fimbriata* cultures from the inoculated test plants further confirmed the Koch postulates, while no *Ceratocystis* isolates were observed in the control seedlings.

Inoculation results showed that the mortality rates of two host plants including *A. heterophyllus* and *A. mangium* were 15-50% and 75-100%, respectively. All inoculated plants exhibited color changes in the sapwood and vascular tissues. The lesion length in each host plant inoculated with all pathogen isolates significantly differed from the control. Specifically, *A. heterophyllus* from 6.9-13.7 cm, *A. muricata* from 9.14-15.9 cm, and *A. mangium* from 9.2-13.46 cm, in comparison to the control at 0.5 cm. Regarding the ITS haplotypes, both ITS5 isolates resulted in similar lesion lengths and mortality rates across all three hosts, while ITS7b exhibited higher lesion lengths and mortality rates compared to the two ITS5 isolates (Table 4).

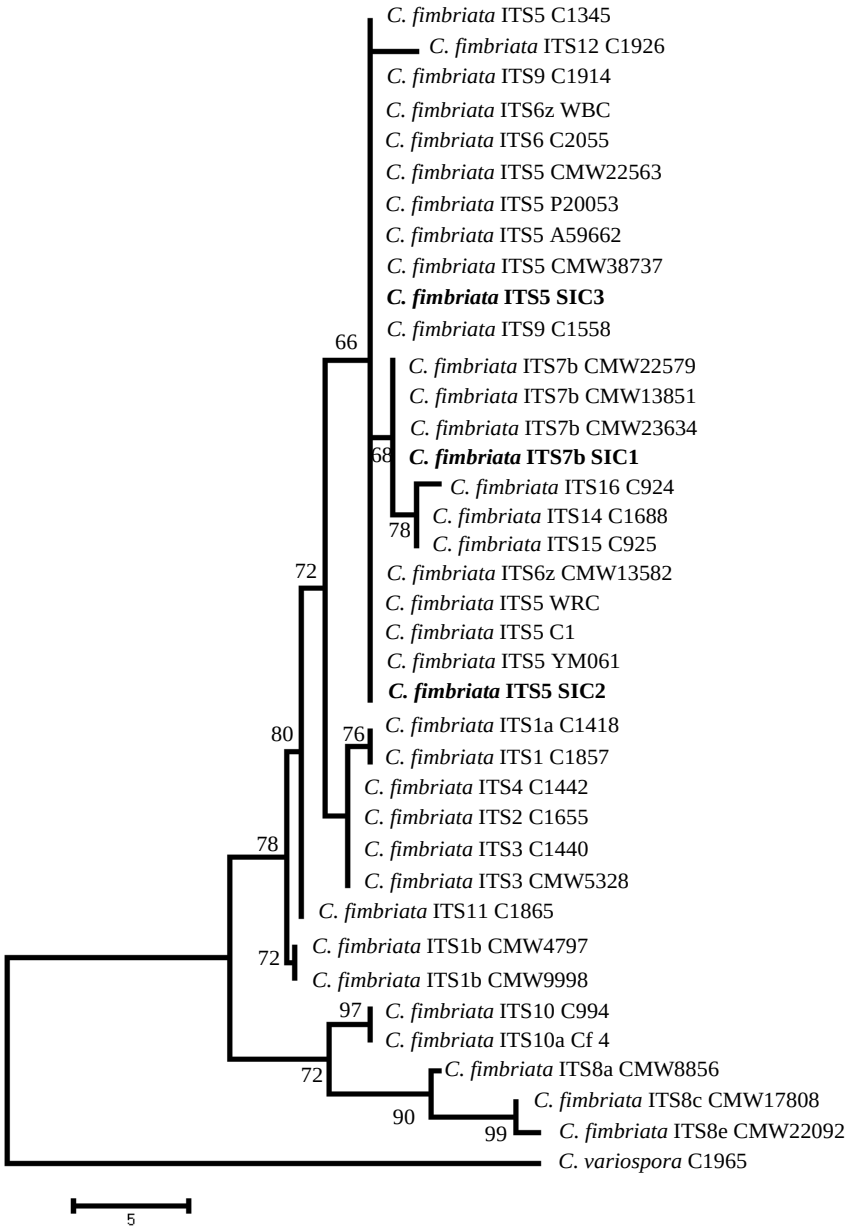

Figure 5. The dendrogram resulting from the maximum parsimony analysis, indicating the genetic linkage of the representative rDNA Isolates obtained from *A. muricata* in Indonesia was highlighted in bold

Table 4. Pathogenicity test of *Ceratocystis* isolates from various plants

Isolates	Host tests	*Artocarpus heterophyllus*		*Annona muricata*		*Acacia mangium*	
		Lesion length (cm)	Wilting and death at 60 dpi[a]	Lesion length (cm)	Wilting and death at 60 dpi	Lesion length (cm)	Wilting and death at 60 dpi
SIC1 (haplotype ITS5)	20	6.9b	3/20	13.9bc	13/20	9.2b	15/20
SIC2 (haplotype ITS5)	20	9.28c	7/20	9.14b	10/20	11.38c	20/20
SIC3 (haplotype ITS7b)	20	13.7d	10/20	15.9c	18/20	13.46d	20/20
Control	20	0.5a	0/20	0.5a	0/20	0.5a	0/20
Fpr		<0.001		<0.001		<0.001	

Notes: According to Tukey's HSD multiple range test, values with the same letters in a column indicated that there were no significant differences among isolates at P=0.05. [a] days post-inoculation (dpi)

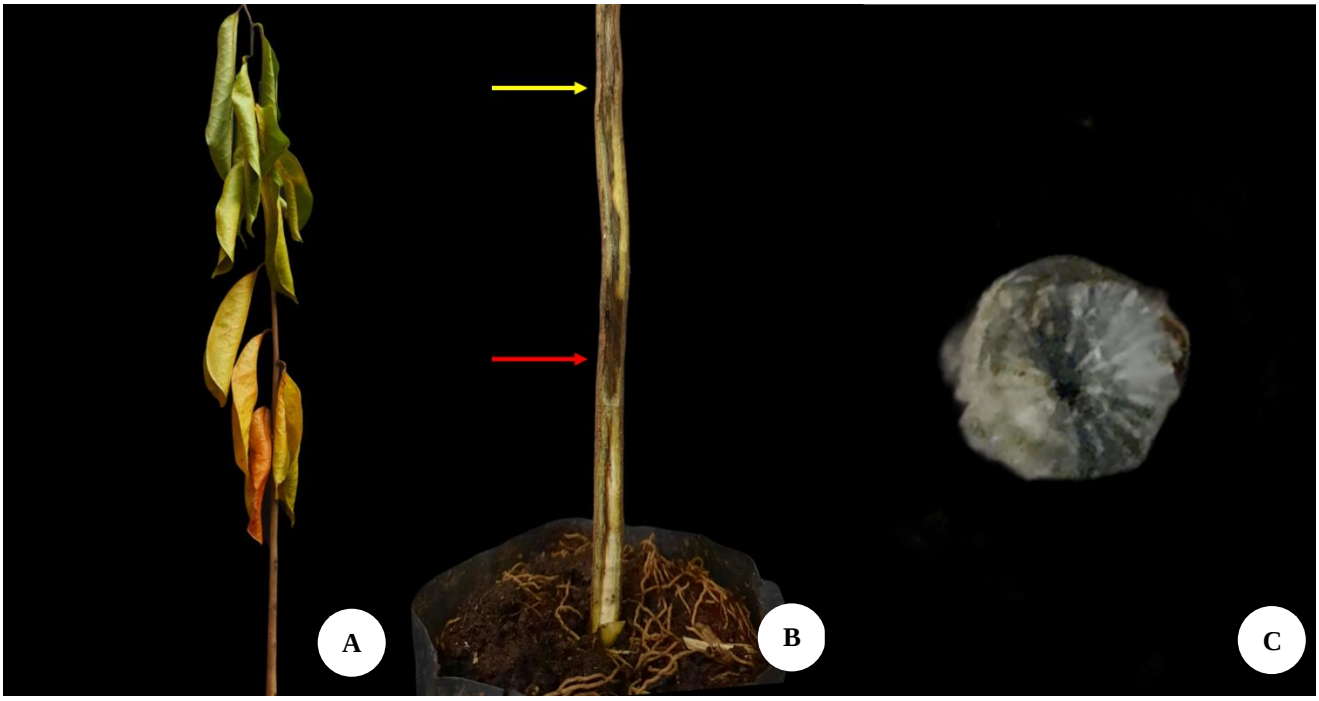

Figure 6. Response of *Annona muricata* seedlings 60 days after under-bark inoculation with mycelium of *Ceratocystis*. A. Total wilting of plant inoculated with SIC3 isolate. B. Yellow arrow indicates the point of inoculation and red arrow the lesion boundary. C. The discolored wood extended to the heartwood of the basal stem of the seedling

Discussion

The present study revealed that *C. fimbriata* is the causative agent of a novel disease affecting soursop plants in plantation areas located in North Indralaya (Ogan Ilir) and certain backyard plants in Gelumbang (Muara Enim), South Sumatra. This is the first recorded case of wilt disease on soursop plants globally, as well as in Indonesia. *C. fimbriata* has been previously reported for infecting *A. mangium* plants in Riau (Tarigan et al. 2011) and Malaysia plantations (Syazwan et al. 2021). Meanwhile, in Indonesia (South Sumatra), it has been associated with wilt disease outbreak in *A.s heterophyllus* (Pratama et al. 2021a), *M. elengi* (Pratama et al. 2021b), and *L. domesticum* (Suwandi et al. 2021; Muslim et al. 2022). *C. fimbriata* is well known for the severe damage caused to various plant families and has a wide host range, such as Myrtaceae represented by *Eucalyptus* (Li et al. 2014); Actinidiaceae represented by *Actinidia* spp. (Piveta et al. 2016); Araceae represented by *C. esculenta* (Oliveira et al. 2017); Meliaceae represented by *L. domesticum* (Suwandi et al. 2021; Muslim et al. 2022); Moraceae represented by *A. heterophyllus*. This is the first report of wilt disease caused by *C. fimbriata* on soursop plants (*A. muricata*) of Annonaceae family in Indonesia.

It was noted that symptoms of *C. fimbriata* infection on *A. muricata* included yellowing leaves, sudden wilting, leaf abscission, branch drying, and a progressive loss of the canopy, ultimately leading to tree death. The infected *A. muricata* trees exhibited typical symptoms of *Ceratocystis* infection, characterized by numerous lesions on the sapwood and the development of lesions on the heartwood, resulting in the occlusion of the vascular tissue of plants. These symptoms closely resembled the serious wilt disease observed in bullet wood trees in Indonesia and Thailand, caused by *Ceratocystis* (Pornsuriya and Sunpapao 2015; Pratama et al. 2021b). Vascular wilting, wood discoloration, and stem canker represented the most characteristic symptoms associated with *C. fimbriata* infection in woody plants (Shukla et al. 2018; Habib et al. 2023). In line with previous studies, root or stem rot, vascular wilting, and canker were common symptoms observed in plants infected with *C. fimbriata* species (Oliveira et al. 2015b; Muslim et al. 2022).

In the present study, the morphological characteristics and gene sequences based on β-tubulin of the three examined isolates exhibited striking similarities to those described for *C. fimbriata* isolated from various host plants worldwide. These included *Coffea arabica* L. in Suriname (Luchi et al. 2013), *Gmelina arborea* in Brazil (Luchi et al. 2013), *Eucalyptus* hybrid in China (Chen et al. 2013), *Acacia crassicarpa* A.Cunn. ex Benth. in Indonesia (Tarigan et al. 2011), *Mangifera indica* in Pakistan and Oman (Naidoo et al. 2013), *L. domesticum* in Indonesia (Suwandi et al. 2021), *Acacia* sp. in Vietnam (Trang et al. 2018), and *E. grandis* in Zimbabwe (Jimu et al. 2015). These *C. fimbriata* isolates collectively constituted the *C. fimbriata* sensu lato (s. l.) complex, with *C. fimbriata* sensu stricto being one of its components (Harrington et al. 2014; Fourie et al. 2015). Based on the gene sequences obtained from ITS analysis, SIC1 was s belonged to the ITS7b haplotype, while SIC2 and SIC3 were classified under the ITS5 haplotype. The dendrogram resulting from the maximum

parsimony analysis, indicating the genetic linkage of the representative rDNA ITS genotype in *C. fimbriata* Sensu stricto and the results of β-tubulin sequence analysis showed that the isolate belonged to the Latin American Clade (LAC). Both isolates from Indralaya (Ogan Ilir) and Gelumbang (Muara Enim) were 99% identical in ITS and β-tubulin sequences, indicating that these isolates represented a single pathogen clone. These results provided strong evidence that soursop wilt disease originated from the introduction of a single haplotype, and its population has spread clonally to infect *A. muricata* trees across the two regions.

Ceratocystis fimbriata has been reported in Indonesia to be associated with wilt and sudden death disease in various plant species, including *Acacia* spp. (Syazwan et al. 2021), bullet wood (Pratama et al. 2021b), and *L. domesticum* (Muslim et al. 2022). The presence of *Ceratocystis* in these plants and the occurrence of the insect carriers *Hypocryphalus mangifera* in the field raised concerns about the potential infection of *A. muricata* with *Ceratocystis* from these sources. *Hypocryphalus mangifera* was a known carriers insect responsible for the worldwide spread of *Ceratocystis* (Al Adawi et al. 2013b; Fourie et al. 2016; Galdino et al. 2017). Furthermore, it has been observed that pruning of *A. muricata* branches using tools previously contaminated by *Ceratocystis* could exacerbate the severity of disease. This was evident from the numerous infected plants found after pruning, using the same tools that were employed to prune infected *Acacia* and bullet wood plants affected by *Ceratocystis*. The severity of disease has been linked to branch wounds resulting from pruning, the timing of pruning, and the pruning techniques used on the plants (Chi et al. 2019). In this study, it was observed that infected *A. muricata* trees were situated near infected *Acacia* and bullet wood, where *H. mangifera* was present in abundance. This suggested that spore contamination might occurred on cutting tools after pruning infected *Acacia* and bullet wood, leading to the spread of spores by the carriers insect *H. mangifera*. Moreover, the use of previously infected *Acacia* and bullet wood land exacerbated the spread of this disease (Souza et al. 2013; Galdino et al. 2017).

The Koch postulates test conducted in the inoculation experiment confirmed that *C. fimbriata* was responsible for the widespread death of soursop trees in South Sumatra. All three isolates showed the ability to infect and kill various plants in the host range test. When *C. fimbriata* isolates were inoculated on *A. heterophyllus*, *A. muricata*, and *Ac. mangium*, these isolates formed lesions on all stems. Imperatively, the most pathogenic isolate was SIC3, which exhibited extensive lesion development and led to high mortality in each plant type. Isolate SIC3, belonged to the ITS7b haplotype. It was known to possess a wide host range and has long been adapted to attack *Acacia* and *L. domesticum* plants in Indonesia (Syazwan et al. 2021; Suwandi et al. 2021; Muslim et al. 2022). The wilt disease caused by *C. fimbriata* in *A. muricata* has rapidly spread to both plantation areas and backyard plants in various regions of Ogan Ilir and Muaraenim District of South Sumatra.

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