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Diversity of *Ceratocystis fimbriata* causing canker and wilt disease on *Cupressus sempervirens* (Italian cypress) in Indonesia

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Abstract. *Pratama R, Suwandi S, Muslim A, Mulawarman. 2025. Diversity of* Ceratocystis fimbriata *causing canker and wilt disease on* Cupressus sempervirens *(Italian cypress) in Indonesia. Biodiversitas 26: 278-287.* In 2022-2023, new diseases were observed on *Cupressus sempervirens* in South Sumatra, Indonesia, with the disease incidence increasing from 17.6% to 26.5% in 2023. Initial symptoms, included stem cankers, black lesions on sapwood and vascular tissue, discoloration and partial wilting of leaves, and eventual complete drying, leading to plant death. The objective of this study was to isolate and identify the fungal pathogen causing wilt disease in *C. sempervirens* trees using morphological characterization and DNA sequencing. In 2022-2023, a disease survey was conducted in six districts of South Sumatra. The results showed that six out of ten locations were infected, with disease incidence ranging from 4.1% to 17.6% in 2022, increasing to 2% to 26.5% in 2023. Pathogen identification employed a polyphasic approach, combining morphological and molecular characteristics from specific genomic regions (the internal transcribed spacer (ITS) and β-tubulin). Both the morphological features (including a globose base with a long neck-ended tip with ostiolar hyphae, cylindrical conidia, and hat-shaped ascospores) and phylogenetic analysis identified the isolates as *Ceratocystis fimbriata.* ITS gene sequences indicated that all the isolates belonged to the ITS5 haplotype. In pathogenicity test, pathogen caused mortality in *C. sempervirens*, *Acacia mangium,* and *Artocarpus heterophyllus* plants. The implications of these findings are significant, as they can potentially lead to the development of effective control measures. To our knowledge, this is the first report of *Ceratocystis* spp. causing wilt disease on *C. sempervirens* in South Sumatra.

Keywords: Canker, *Ceratocystis fimbriata*, *Cupressus sempervirens*, sap wood, wilt disease

INTRODUCTION

Cupressus sempervirens L., commonly known as the Mediterranean cypress or Italian cypress, is a popular ornamental tree species found in many parts of the world. It is an iconic evergreen tree species that spread in horticulture and landscaping areas, significantly. Native to regions surrounding the Mediterranean Sea, it has been admired for centuries and cultivated for its tall, slender form, timeless elegance, and cultural symbolism (Orhan and Tumen 2015). *C. sempervirens* belongs to the Cupressaceae family and is characterized by its tall, columnar shape with dense, dark green foliage. It typically reaches heights of 40 to 60 feet (12 to 18 meters) or more. The tree's scale-like leaves are dark green and aromatic when crushed, exuding a pleasant, resinous scent. The leaves of *C. sempervirens* are typically slender and pointed, forming compound structures and are bright green when young, turning dark green as they age (d'Auria et al. 2020). The young leaves of cypress are about 1 to 1.5 cm long by approximately 1 mm in diameter. Pressed closely to the twigs, they look in cross-section as dense scale-like masses 2-5 mm long (Roman et al. 2016).

In Indonesia, *C. sempervirens* trees were mainly found in the cooler highlands of Java, Bali, and Sumatra. These trees, known for their tall and narrow shape, are often planted in parks and gardens. While valued for its drought resistance and ability to grow in well-drained soils, Indonesia's tropical climate is not ideal for its growth. *C. sempervirens* is an ornamental plant and street shade in South Sumatra, Indonesia. It is used for various medicinal purposes. The essential oil in the nose and throat areas, where it is commonly used as an aid to natural respiration practices. The essential oil is used for respiratory problems, especially in the nose and throat areas as an aid in natural respiration. It is opined to have decongestant activities that relieve symptoms of respiratory illnesses such as bronchitis, colds, and coughs (Batiha et al. 2023). It also relaxes and relieves stress (Galovičová et al. 2023). Cypress essential oil is reported to have antimicrobial activity, inhibiting the growth of certain bacteria and fungi with a similar effect (Nouri et al. 2015; Pansera et al. 2023). Its oil can be added to soaps and other skincare products designed for oily skin or acne because of its astringency (Khan et al. 2017).

Among the several diseases and pests affecting *C. sempervirens*, cypress canker caused by *Seiridium cardinale* predominates, which is evidenced mainly in symptoms of branch dieback with foliage yellowing to browning followed by resin-flow from cankers on stems and branches (Milenković et al. 2022). Branch death, wilting, and browning are associated with *Cypress* blight caused by *Seiridium* spp. (Danti and Rocca 2017). *Phytophthora* root rot caused by *Phytophthora austrocedri*, produces symptoms, such as reduced growth rates, root necrosis, phloem lesions, and chlorosis of leaflets in the moderate phase cut followed occasionally by dead branches (Mahdikhani et al. 2017). A

new disease was observed during August 2021 on existing trees of *C. sempervirens* in many cities and districts of South Sumatra, causing yellowing leaves followed by drying to total plant death. Based on the symptoms and identification the fungi was identified as *Ceratocystis*, which causes wilt disease in plants. Similar disease attacks have been reported on agroforestry crops in South Sumatra, such as lethal wilt on *Lansium* tree (Suwandi et al. 2021; Muslim et al. 2022) caused by *Ceratocystis fimbriata*, sudden decline disease on bullet wood (*Mimusops elengi*) caused by *Ceratocystis manginecans* (Pratama et al. 2021a), wilt and sudden death on *Artocarpus heterophyllus* (jackfruit) (Pratama et al. 2021a), and wilt disease on *Annona muricata* (soursop). The objective of this study was to isolate and identify the fungal pathogen causing wilt disease in *C. sempervirens* trees using morphological characterization and DNA sequencing. Sap stain fungi were identified based on their morphological characters and molecular data from infected wood of Italian cypress.

MATERIALS AND METHODS

Sample collection and fungal isolation

The disease survey was conducted from August 2022 to September 2023 and sampling was done in the districts of Ogan Ilir, Ogan Komering Ilir, Musi Banyuasin, Muara Enim, Palembang City, and Prabumulih, South Sumatra Province, Indonesia. *C. fimbriata* was collected from newly infected wounds on *C. sempervirens*, which showed symptoms of branch wilting, vascular tissue discoloration, and plant death. Diseased plants at each location were observed, and their incidence was calculated using the following formula:

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

Samples were obtained by making bark incisions and cutting approximately 50 mm tangential longitudinal sections of the freshly infected xylem with sap stain. The plants were between 5 and 10 years old. The wilt disease symptoms, included assessing lesion development, bark and wood discoloration, resin flow from the lesion surface, leaf wilting or shedding, and tree mortality. Wood samples were placed in plastic bags and kept in refrigerator until isolation. Discolored wood pieces were first sterilized with sodium hypochlorite (NaOCl) for five minutes, rinsed with distilled water, and then dried in a laminar airflow, were sandwiched between two carrot discs. The carrot discs were incubated at room temperature for 5 to 10 days to induce fungal sporulation (Piveta et al. 2016; Brito et al. 2019). Mycelium grown from woody slices of diseased plants was transferred to new Petri dishes containing 2% malt extract agar (MEA, 20 g/L malt extract, 20 g/L agar) and incubated in darkness at 25°C.

Morphological characterization

The morphological characteristics of *C. fimbriata*, isolated from *C. sempervirens* trees, were compared with two isolates from Palembang (CLC1 and CLC4), two from Indralaya, Ogan Ilir (CLC3 and MHC6), and two from Prabumulih (CLC2 and CLC5). The macroscopic evaluation, included colony color, shape, and margins as the isolates grew. Microscopic measurements were made on 14-day-old cultures using an Olympus Cx33 microscope and Sigma MTN020 camera, and the images were processed using Images-Pro Plus software (Media Cybernetics, Inc). Ascospores were measured at 1000× magnification, chlamydospores, cylindrical conidia, and barrel conidia at 400× magnification, and perithecia at 100× magnification. Morphological measurements were based on 100 replicates for each isolate (Pratama et al. 2021a).

DNA extraction, amplification, sequencing, and phylogenetic analysis

For DNA isolation, MEA cultures were transferred to potato dextrose broth (PDB) media and incubated at 28°C for 12 days. The mycelium from the PDB culture was filtered, dried, and finely powdered using a mortar and pestle. DNA extraction was carried out with the Zymo Research ZR Fungal/Bacterial DNA MiniPrep™ kit (Irvine, California, USA). Then, a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) was used to determine the DNA purity and concentrations.

PCR amplification and sequencing were targeted at two gene regions, namely beta-tubulin, using the primers βT1a: TTCCCCCGTCTCCACTTCTTCATG and βT1b: GACGAGATCGTTCATGTTGAACTC, as described by (Thu et al. 2024), and the internal transcribed spacer (ITS), using the primers ITS1: 5k TCC GTA GGT GAA CCT GCG G 3k and ITS4: 5k TCC TCC GCT TAT TGA TAT GC 3k, according to (Yunus et al. 2024). Amplification was carried out in 50 µL reactions consisting of 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 of template DNA, and 27 µL of sterile water. PCR was conducted using a C1000 Touch™ thermal cycler (Bio-Rad, USA). The PCR cycle parameters were as follows: an initial denaturation for 3 min at 94°C, followed by 30 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min. Amplification was concluded at 72°C for 10 min, and PCR products were stored at 10°C. Subsequently, PCR amplicons were sequenced at 1st BASE (Malaysia). The DNA sequences were compared with the GenBank database using a nucleotide-nucleotide BLAST search at the National Center for Biotechnology Information, Bethesda, USA. Relevant sequences were transferred and processed using BioEdit software (Pratama et al. 2021b).

Phylogenetic trees were generated and edited in MEGA v.7 using maximum parsimony (MP) analysis with 1,000 bootstrap replicates (Kumar et al. 2016). Node support was determined by performing 1,000 bootstrap replicates on aligned sequences. The metrics calculated for MP analysis included tree length, retention index (RI), and consistency index (CI). *Ceratocystis virescens* served as the outgroup taxon, and the in-group was considered to be monophyletic.

Koch's postulate and host range test

The pathogenicity evaluation involved two stages using six isolates (CLC1, CLC2, CLC3, CLC4, CLC5, and CLC6). To confirm Koch's postulates, 7-month-old *C.*

sempervirens plants were used for the first inoculation test. 10 host plants were inoculated with each *Ceratocystis* isolate, and an equal number of seedlings were inoculated with sterile MEA as a control. The plants were collected with a stem diameter of 2-3 cm and a height of 60-70 cm from local seedlings and, for the experiment, placed in 15 cm diameter pots containing peat soil. Each *C. sempervirens* plant was wounded with a sterile scalpel by making a 10 mm-long incision on the stem, approximately 20 cm above the soil surface. Then, agar mycelium (4 mm diam) was inserted into each wound site. All the inoculated wounds were covered with moistened sterile cotton and sealed with parafilm. The inoculated plants were put in the greenhouse and watered twice daily. After 90 days, bark tissue from the inoculated seedlings was incised at both the top and bottom of the inoculation site, and the length of the lesion was measured (Muslim et al. 2019; Pratama et al. 2023a). Next, to re-isolate the inoculated pathogen, wood samples were obtained from the lesion's edge and cultured on MEA plates or sandwiched between two carrot slices.

In the second inoculation test, the pathogenicity of isolates was examined against two agroforestry plants, namely *Acacia mangium* and *Artocarpus heterophyllus.* The plants used were five months old, with a stem diameter of 2-3 cm and a height of 60-70 cm, all grown in the same potting medium as described in the first experiment. These plants were maintained under experimental house conditions and watered twice daily. Inoculation was conducted using the same number of isolates and the same procedure as described in the first experiment.

Data analysis

The pathogenicity test data were analyzed using the SAS University Edition software package. Tukey's honestly significant difference (Tukey's HSD) test and analysis of variance (ANOVA) determine the significant differences in the means of the different treatments.

RESULTS AND DISCUSSION

Symptoms and incidence of wilt disease on *Cupressus sempervirens*

Cupressus sempervirens plants were commonly planted along roads, city parks, and house yards in various cities and districts of South Sumatra. The symptoms of *Ceratocystis* infection in *C. sempervirens* were formation of canker on the stem, leading to the wilting and drying of leaves (Figure 1.A). Gradually, pathogen spreads throughout the stem sapwood, causing blackish-colored stem bark and long, irregular black lesions when cut (Figure 1.B). Ultimately, the pathogen progresses into the plant's vascular tissue, resulting in discolored, blackish-brown vascular tissue that functions inadequately (Figure 1.C). It was also observed that the leaves of the affected plants changed color from green to yellow-brown and dried up. In the later stage, the leaves drop off, leaving only the branches and stem, ultimately leading to the plant's demise.

In 2022-2023, disease survey was done in six districts of South Sumatra, Indonesia the results of which revealed that six out of 10 locations were infected by *Ceratocystis* (Table 1). In Palembang City's Seberang Ulu I Sub-district, specifically Jakabaring, the incidence increased from 7.4% in 2022 to 14.8% in 2023. Incidence in Kertapati increased from 4.1% in 2022 to 9.1% in 2023. Ilir Barat I Sub-district, including Bukit Siguntang and Danau Kembang Demang, was initially unaffected, but saw an increase to 16.7% in 2023. Conversely, the Alang-Alang Lebar Sub-district, especially the Punti Kayu tourist park, recorded a 0% incidence, with no impact on *C. sempervirens* plants, providing a stark contrast to the areas with high disease incidence. In Prabumulih City, specifically the Lembak Area (North Prabumulih), infected *C. sempervirens* plants increased from 4.6% in 2022 to 7% in 2023. In Muara Enim district, particularly Gelumbang, the disease incidence rose from 0% in 2022 to 2% in 2023. Ogan Ilir, notably the Sriwijaya University Farm area, experienced an increase from 17.6% in 2022 to 26.5% in 2023. Ogan Komering Ilir District (Celikah and Tanjung Rancing) and Musi Banyuasin District (Sekayu) showed 0% incidence.

Table 1. Wilt disease incidences in *Cupressus sempervirens* plants in South Sumatra

Locations	District/cities	Disease incidence (%)	
		2022	2023
Jakabaring	Palembang	7.4 (2/27)*	14.8 (4/27)
Kertapati	Palembang	4.1 (4/99)	9.1 (9/99)
Bukit Siguntang	Palembang	0 (0/12)	16.7 (2/12)
Punti Kayu	Palembang	0 (0/24)	0 (0/24)
Lembak	Prabumulih	4.6 (5/115)	7.0 (8/115)
Gelumbang	Muara Enim	0 (0/101)	2.0 (2/101)
UniversitasSriwijaya Farm	Ogan Ilir	17.6 (6/34)	26.5 (9/34)
Celikah	Ogan Komering Ilir	0 (0/46)	0 (0/46)
Tanjung Rancing	Ogan Komering Ilir	0 (0/106)	0 (0/106)
Sekayu	Musi Banyuasin	0 (0/583)	0 (0/583)

Notes: (*): Disease incidence (%), number of diseased plants/total observed plants

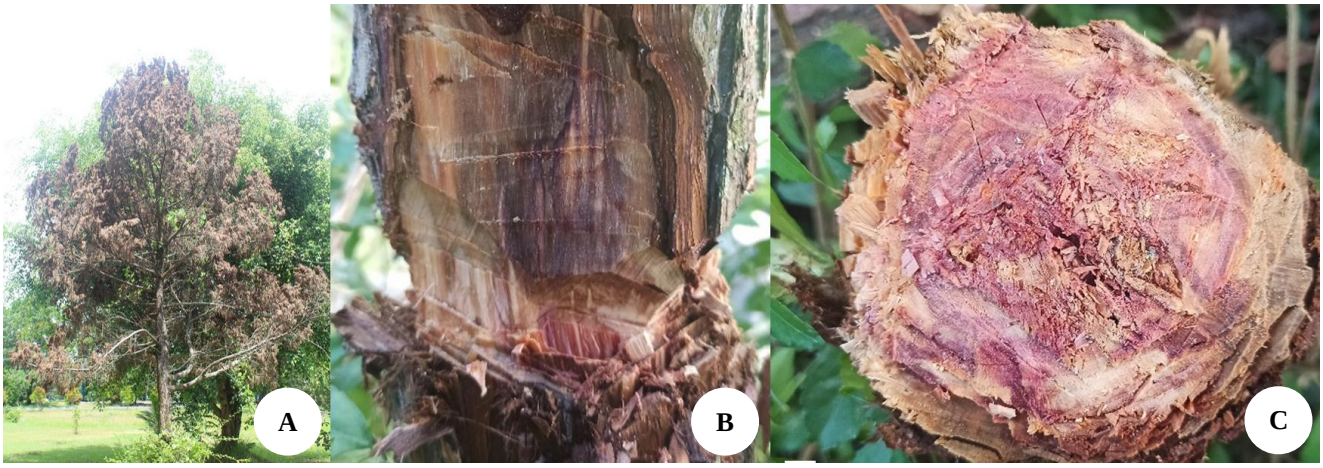

Figure 1. The wilt disease symptoms in *Cupressus sempervirens* plants. A. Wilting and dying of *C. sempervirens* plant; B. Formation of black-colored lesions in the sapwood tissue of infested *C. sempervirens* plants; C. Development of pathogen infestation in the vascular tissue of *C. sempervirens* plants

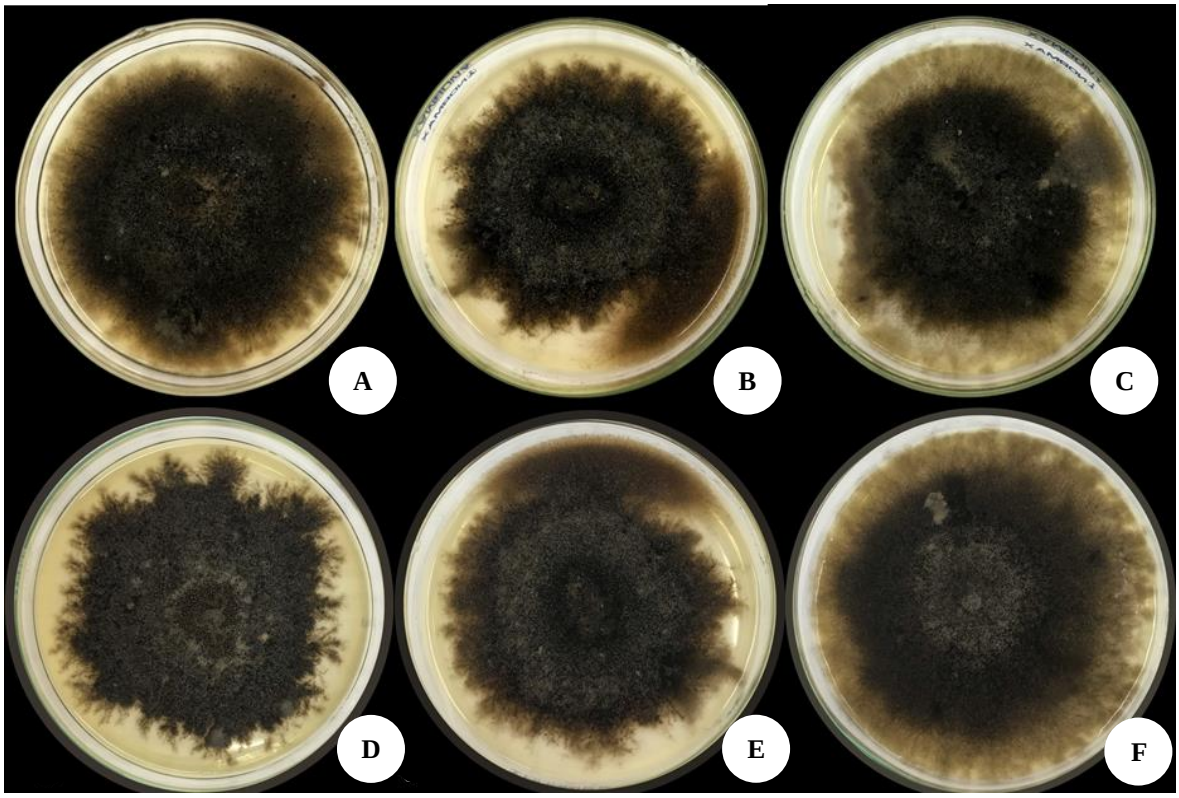

Figure 2. Isolates of *Ceratocystis fimbriata* on MEA medium: A. CLL1; B. CLL2; C. CLL3; D. CLL4; E. CLL5; and F. CLL6

Morphological characterization

All of the isolates cultivated on MEA exhibited uniform morphological characteristics, producing an impressive abundance of conidia, ascospores, chlamydospores, and perithecia. Colonies were irregular with undulate margin shapes. The mycelium was light gray to dark gray, or greenish color and the reverse side of all isolates was black. Notably, isolates CLL1 and CLL6 demonstrated relatively faster growth, and covered the whole culture of Petri dishes (9 × 15 cm) within 25 days, while other isolates required 40 days (Figures 2.A-2.F). There were no notable variations in structural dimensions among all the isolates, encompassing ascomata, ascospores, and chlamydospores.

All the isolates exhibited dark brown to black globose to subglobose ascomatal bases, with dimensions ranging from 127.8-239.3 x 104.7-229.4 µm (Figure 3.A). The ascomatal necks were typically erect, occasionally curved, spanning from 317.5 to 572.7 µm, and ostiolar hyphae diverged at the tip, releasing cap-shaped ascospores (Figure 3.B). Phialides displayed a pale brown to hyaline color

(Figure 3.C). Oval-shaped chlamydospores were present in all isolates, characterized by thick-walled, smooth, and sometimes aggregated structures with a chain-like form, measuring 5.8-14.5 x 5.1-11.8 µm (Figure 3.D-3.E). Ascospores exhibited a cap-like shape, measuring 2.8-5.9 × 2.1-5.5 µm (Figure 3.F). The conidia were barrel shaped measured 3.9-14.8 x 2.3-6.1 µm (Figure 3.G), while bacilliform conidia measured 10.6-34.7 x 1.7-6.5 µm (Figure 3.H). Based on the macroscopic and microscopic characteristics, all the observed isolates were similar to those of *C. fimbriata*.

Phylogenetic analysis

All the isolates were kinship-determined by manually sequencing ITS and aligning them with ITS haplotypes using reference ITS genotypes. MP analysis revealed that all the obtained isolates belonged to *C. fimbriata* ITS5 haplotype (Figure 4), with a CI of 0.580645, RI of 0.857143, and CoI of 0.680272.

The β-tubulin dataset comprised ex-type and ex-paratype sequences representing species within the Latin American (LAC) and Asian clades of the *C. fimbriata* species complex. β-tubulin MP analysis was conducted using MEGA 7 with 1000 replicates. All *Ceratocystis fimbriata* isolates obtained were classified within the LAC of *C. fimbriata* sensu lato (Figure 5). Based on the phylogenetic results, these isolates were closely related to ex-type and ex-paratype *C. manginecans* and *C. fimbriata*. Presently, *C. manginecans* was considered synonymous with or a type of *C. fimbriata* sensu stricto, with a CI of 0.933333, RI of 0.977099, and CoI of 0.933349.

Koch's postulate and host range test

The results of pathogenicity test demonstrated that all the isolates were capable of infecting and inducing disease symptoms similar to field attacks (Figure 6). Lesion length measurements revealed that isolates CLC2 and CLC3 exhibited strong pathogenicity, with lesions measuring 4.23 to 4.8 cm and resulting in 30-50% plant mortality. In contrast, isolates CLC1, CLC4, CLC5, and CLC6 displayed lower pathogenicity, generating lesions measuring 3.42 to 4.05 cm, with a consistent 10% plant mortality rate (Table 2).

Host range test using *A. mangium* and *A. heterophyllus* revealed that all isolates could infect plant hosts other than *C. sempervirens* causing lesion formation, wilting and plant mortality. All isolates were highly pathogenic on *A. mangium* plants, producing lesion length of 7.72-11.26 cm and end-point mortality ranging from 60 to 100%. All the isolates produced lesions on pathogenicity tests carried out on *A. heterophyllus* plants of size ranging from 6.18 to 10.30 cm, with a plant mortality rate between 10-50%.

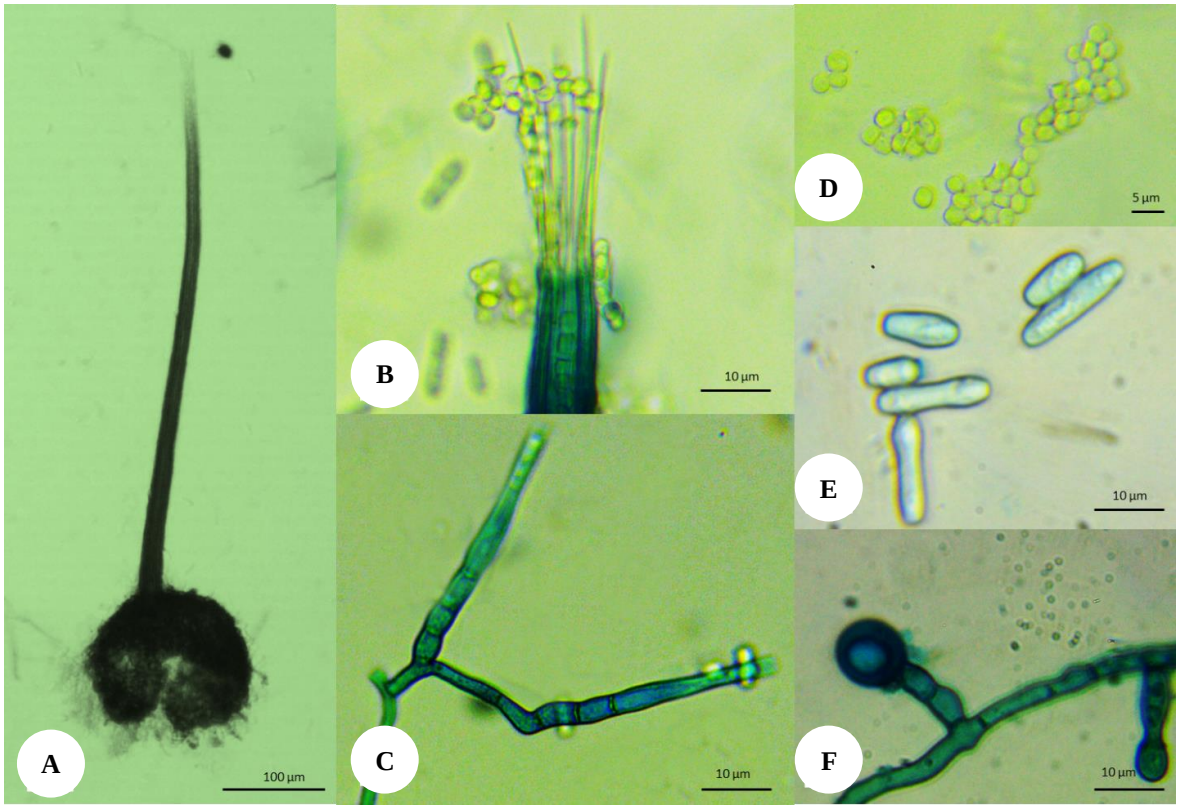

Figure 3. Microscopic features of *Ceratocystis fimbriata*: A. Ascoma; B. Divergent ostiolar hyphae; C. Flask-shaped primary phialide-producing cylindrical conidia; D. Clustered ascospores; E. Cylindrical conidia; F. globose chlamydospores

Table 2. Plant mortality and lesion length observed 90 days after pathogen inoculation

Isolates	*Cupressus sempervirens*		*Acacia mangium*		*Artocarpus heterophyllus*	
	Lesion length (cm)	**Dead plants**	**Lesion length (cm)**	**Dead plants**	**Lesion length (cm)**	**Dead plants**
CLC1	3.87bc*	1/10	9.39de	8/10	7.20cd	3/10
CLC2	4.23cd	3/10	9.97e	10/10	7.67d	3/10
CLC3	4.80d	5/10	11.26f	10/10	10.30e	5/10
CLC4	4.05c	1/10	8.74cd	8/10	6.40b	3/10
CLC5	3.42b	1/10	7.72b	6/10	6.18b	1/10
CLC6	3.83bc	1/10	8.12bc	6/10	6.73bc	3/10
Control (MEA)	0.5 a	0/10	0.5a	0/10	0.5a	0/10
P	<0.001		<0.001		<0.001	

Notes: *: a-f (different superscripts in each row indicate significant ($p<0.05$) differences)

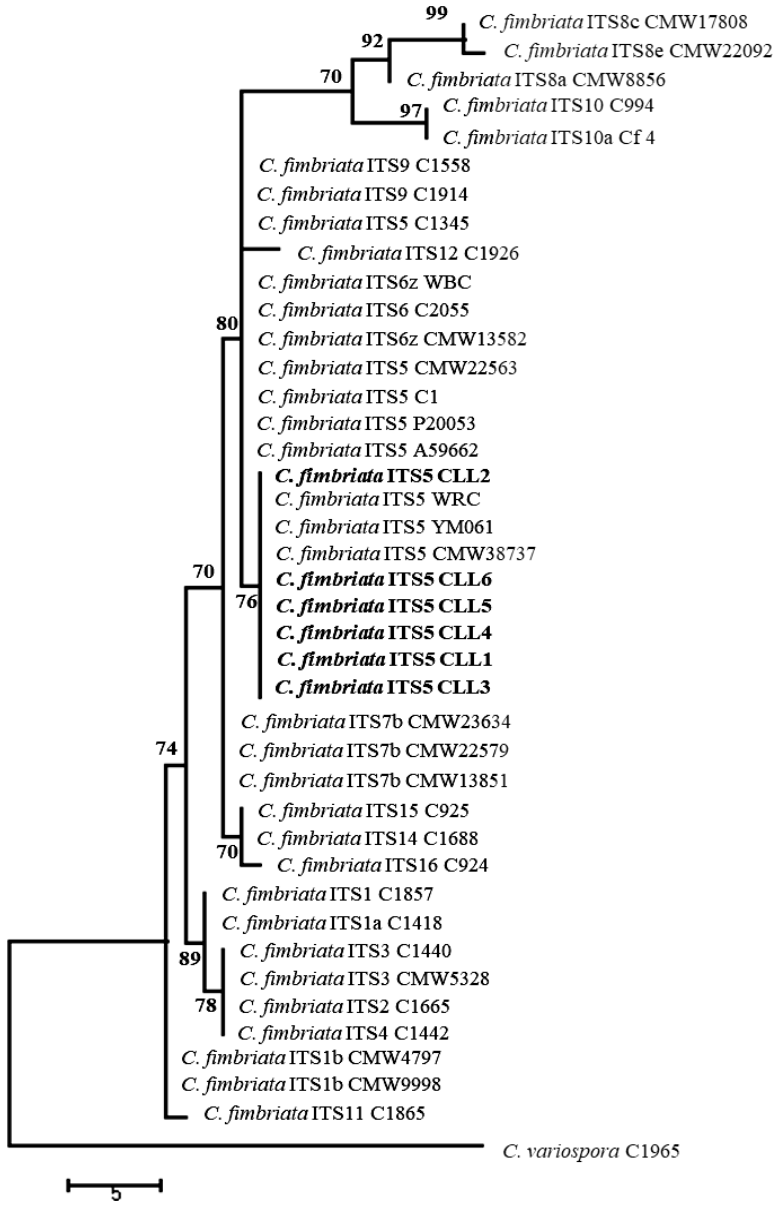

Figure 4. A phylogenetic tree, generated by MEGA using MP analysis, including the ITS sequences of all isolates (highlighted in bold) and the genotypes of *Ceratocystis fimbriata* sensu stricto

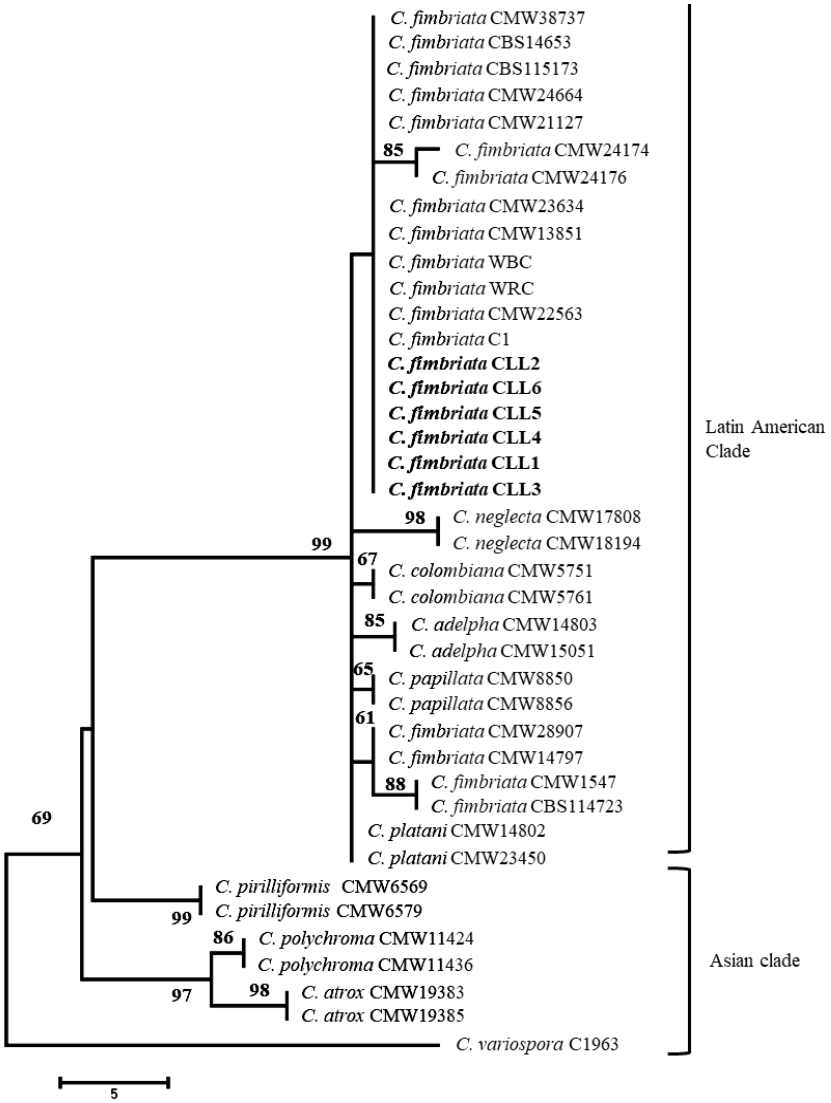

Figure 5. A MEGA-generated phylogenetic tree using MP analysis, incorporating β-tubulin sequences from all the isolates (highlighted in bold) and other species within the LAC and Asian clade of the *Ceratocystis fimbriata* species complex

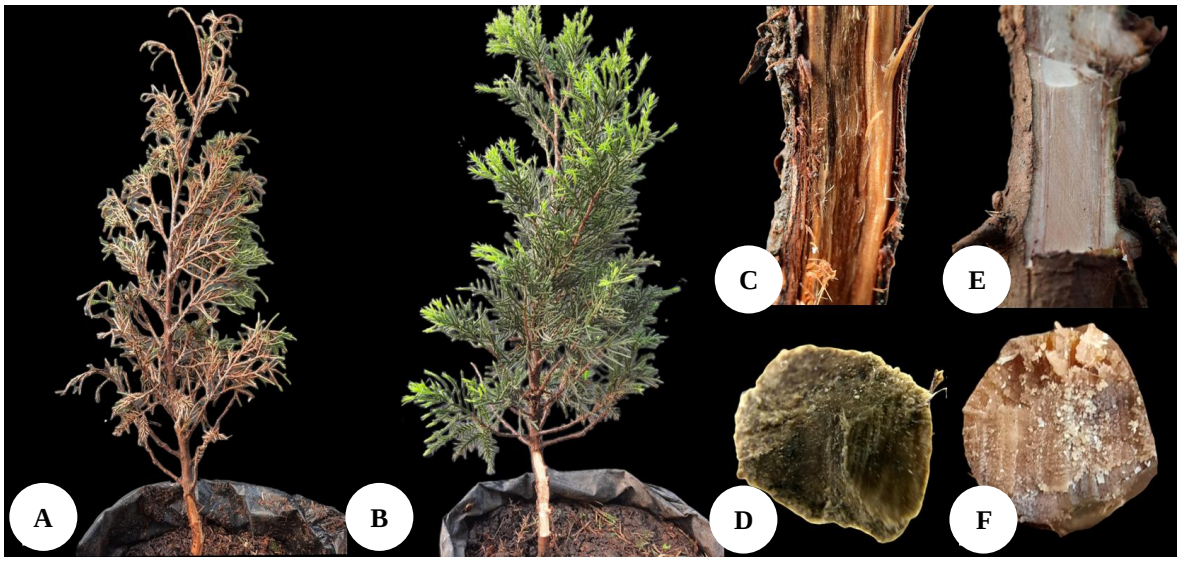

Figure 6. The wilt disease symptoms in *Cupressus sempervirens* plants on pathogenicity test: A. Wilting and dying of *C. sempervirens* plant; B. Healthy control seedlings of *C. sempervirens*; C-D. Formation of black-colored lesions in the sapwood and vascular tissue; E-F. No formation of black-colored lesions in the sapwood tissue and vascular tissue on control seedlings

Discussion

In 2022, wilt on *C. sempervirens* was observed in four locations and then spread to six locations across six districts. This wilt disease spreads rapidly, causing wilting, necrosis of the sapwood and heartwood tissues, and sudden plant death, posing a serious threat to *C. sempervirens* plants. Disease incidence increased by approximately 200% between 2022 and 2023. The outbreak was confined to six out of 10 locations in these districts and cities. The limited extent of the spread was due to the early stage of the outbreak and the unfavorable conditions for the pathogen attack, including pathogen virulence, weather, wind, and beetles as disease vectors. During the site survey, *Acacia* plants were also found infected with *Ceratocystis* wilt, serving as a potential source of inoculum. The symptoms of canker disease in *C. sempervirens* were similar to the symptoms of *C. fimbriata* wilt disease described in West Indian mahogany (Muslim et al. 2025), *Annona muricata* (Pratama et al. 2023a), *A. heterophyllus* (Pratama et al. 2021b), *Mangifera indica* (Pratama et al. 2023b), *Acacia crassicarpa* and *A. mangium* species in Sabah, Malaysia (Syazwan et al. 2022; Amelia et al. 2024; Yunus et al. 2024), and *Eucalyptus* in China and Pakistan (Li et al. 2014; Liu et al. 2015; Alam et al. 2017). The symptoms in roots are also similar to the symptoms of *C. fimbriata* wilt disease described in *L. domesticum* species in Indonesia (Muslim et al. 2022).

All the isolates matched *C. fimbriata* in morphology. The morphological characteristics of the isolates in this study closely match the descriptions provided for *C. fimbriata* isolated from jackfruit trees (Pratama et al. 2021b), as well as *L. domesticum* (Suwandi et al. 2021; Muslim et al. 2022). They had ITS rDNA sequences, placing the pathogen in the ITS5 haplotype of *C. fimbriata*, alongside isolates from *A. crassicarpa, A. mangium*, and *Colocasia esculenta* (Li et al. 2016; Syazwan et al. 2022). Using ITS rDNA sequences to define species within the *C. fimbriata* complex has been challenging due to the prevalence of insertions and deletions found among and within pathogen populations (Harrington et al. 2014; Oliveira et al. 2017). Some of these isolates were previously identified as *C. acaciivora* (Syazwan et al. 2022) and later reconsidered as *C. manginecans* (Fourie 2015). However, Fourie et al. (2015) and Li et al. (2016) regarded the cryptic species as a synonym or conspecific of *C. fimbriata* sensu stricto. β-tubulin sequences place the pathogen within LAC genera. Three species names are associated with the pathogen: *C. manginecans*, *Ceratocystis acaciivora*, and *C. fimbriata*. In this context, the name *C. fimbriata* was employed to identify the pathogen responsible for causing wilting and death in *C. sempervirens* plants. This aligns with Oliveira et al. (2015) findings, indicating that *C. manginecans* and *C. acaciivora* are not distinct species. β-tubulin sequencing revealed no nucleotide sequence differences between these two species and *C. fimbriata*. The disparity in the ITS primers signifies a limited variation in the DNA sequence, classifying *C. manginecans* and *C. acaciivora* as genotypes of *C. fimbriata* sensu stricto. Phylogenetic analyses using ITS regions and β-tubulin definitively identified the *Ceratocystis* isolate causing canker and wilt in Italian cypress in Indonesia as *C. fimbriata* sensu stricto.

Pathogenicity test confirmed that *C. fimbriata* was able to cause infection in *C. sempervirens* tree. Pathogenicity tests on the other two plants showed that all six isolates infected *A. mangium* and *A. heterophyllus* plants and formed stem lesions, leading to the death. Isolate CLC3 was the most pathogenic, resulting in extensive lesion development and high plant mortality 90 days post-inoculation. All the isolates belonged to the *C. fimbriata* ITS5 haplotype, known for causing lethal wilt diseases in economically significant crops globally. *C. fimbriata* is notorious for its devastating impact on various plant families, with a broad host range, including Myrtaceae (*Eucalyptus*) (Li et al. 2014), Actinidiaceae (*Actinidia* spp.) (Piveta et al. 2016), Annonaceae (*A. muricata*) (Pratama et al. 2023a), Araceae (*C. esculenta*) (Oliveira et al. 2017), Meliaceae (*L. domesticum*) (Suwandi et al. 2021; Muslim et al. 2022), Moraceae (*A. heterophyllus*) (Pratama et al. 2021b). This study confirms *C. fimbriata's* ability to kill *C. sempervirens*, a Cupressaceae family member in Indonesia, underscoring its wide host range and potential to infect other trees. To our knowledge, this is the first report of *Ceratocystis* spp. on *C. sempervirens* in South Sumatra, Indonesia.

C. fimbriata in Indonesia has probably expanded its host range from *A. crassicarpa* and *A. mangium* in Riau, Indonesia to *A. heterophyllus, L. domesticum*, and *M. indica* in South Sumatra, Indonesia. Pathogenicity tests confirmed that *A. mangium* and *A. heterophyllus* were more susceptible than the original host (*C. sempervirens*), demonstrating *C. fimbriata's* pathogenicity on *Acacia* and *A. heterophyllus* as primary hosts. Similar disease symptoms caused by *Ceratocystis* infection were endemic in *Acacia* and *A. heterophyllus* plantations located 3-5 km from the study site. Pathogenic *C. fimbriata* populations in *Acacia* plantations could potentially extend their host range to *C. sempervirens* trees, posing a serious threat to neighboring agroforestry tree species. In Indonesia, the ITS5 haplotype of *C. fimbriata* has expanded its host range to susceptible neighboring crops. Genotypes of *L. domesticum* have exhibited strong aggressiveness on *A. heterophyllus* (Pratama et al. 2021b) and led to epidemics of bullet wood (Pratama et al. 2021a). A similar host expansion involving the ITS5 haplotype has occurred in China, where eucalyptus populations have caused epidemics among pomegranate, loquat, and taro (Harrington et al. 2015; Li et al. 2016), as well as tea trees (Xu et al. 2019).

The most prevalent type of disease in *C. sempervirens* is very close to the wood borer *Hemicriconemoides mangiferae*, which chews many holes in the stems and is a confirmed carrier for *Ceratocystis* disease of mango plants in Oman and Pakistan (Al-Adawi et al. 2013). Field observations showed many unhealthy plants, several of which were the result of branch pruning. This led to scars on the plant, which provided a perfect entrance point for *Ceratocystis*. Poor disinfection measures after pruning contaminated tools used in the field had the effect of making matters even worse in this regard (Chi et al. 2019). The high number of infected wild *Acacia* plants near *C. sempervirens* may facilitate the transmission of *Ceratocystis*

disease (Muslim et al. 2022). Notably, no wounds from wild vertebrates like squirrels or macaques, previously reported as significant disease spreaders, were observed in the field (Nasution et al. 2019; Suwandi et al. 2021). In conclusion, *C. fimbriata* was found to be associated with canker and wilt disease in *C. sempervirens* in Indonesia. Pathogenicity test confirmed that all the isolates have the potential to cause mortality in plants. Host range test using *A. mangium* and *A. heterophyllus* revealed that all isolates could infect plant hosts other than *C. sempervirens*. This information provides early insights into the occurrence of this plant pathogen and serves as a precautionary measure for future forest plantation stakeholders.

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