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First report of new wilt disease on *Mangifera indica* caused by *Ceratocystis fimbriata* in Indonesia

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Abstract

Ceratocystis wilt and canker disease has severely compromised the profitability of Mangifera indica plantations in the world. In 2022, wilt and sudden death were observed on Mangifera indica. Identification was performed by sequence analysis of the concatenated β-tubulin gene regions. Sequencing of the PCR product confirmed this pathogen was Ceratocystis fimbriata sensu stricto. This is the first report of C. fimbriata causing sudden death disease in M. indica in Indonesia.

Keywords: first report, Wilt Ceratocystis, Manggo, South Sumatera, Indonesia

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1. Introduction

*Mangifera indica*belongs to the family Anacardiaceae, and it is known in the world as mango and in Indonesia as Mangga. The mango plant is an annual fruit plant in the form of a tree that originated in India. This plant then spread to Southeast Asia including Malaysia and Indonesia [1]. The mango tree is a higher plant whose stem structure includes the arboreus group, which is a woody plant that has a stem height of more than 5 m. Mango can reach 10-40 m in height. Mangoes can reach a height of 10-40 m. Mango grows in the form of an upright, multi-branched tree, and has a green leafy crown throughout the year. The height of a mature tree can reach 10-40 m [2]. The age of the tree can reach more than 100 years. Mango plants have many benefits as the fruit is high in Vitamin C, mango leaf shoots a decrease in blood glucose levels, mango leaves have contents that are beneficial to health, such as flavonoids, phenols, tannins, terpenoids, and quinones can function as anti-inflammatory [3];[4].

Wilt disease caused by *Ceratocystis* was first reported in Oman. Epidemics of this disease are very serious and cause sudden decline, having caused the death of thousands of trees in Oman. The disease was first discovered in 1998 in the Barka area in the southern part of Al Batinah region [5]. The cause of the disease is known to be a virulent fungal pathogen, *Ceratocystis manginecans* [6]; [7]. In Oman, the bark beetle *Hypocryphalus mangiferae* is closely associated with trees affected by the mango decline disease caused by *Ceratocystis manginecans*. In 2021, wilt and die-back symptoms were observed for the first time in *M. indica* plants at Sriwijaya University field, Indralaya, South Sumatra, Indonesia.

Ceratocystis fimbriata is a pathogen that has currently infected many plants in Indonesia, especially in South Sumatra. This pathogen has been reported to infected *L. domesticum* plants in Ogan Komering Ulu in 2015 [8] and currently the pathogen has spread widely throughout the duku plantation area in South Sumatra [9]. This pathogen has also been reported to cause sudden decline and sudden death in jackfruit [10] and Bullet Wood [11]. The objective of this study was to identify the causal agent of the sudden decline of disease in *M. indica*. Sap stain fungi were isolated from infected *M. indica* and identified based on morphological characteristics and molecular analysis.

2. Materials and Methods

2.1 Disease Surveys

Disease surveys were conducted between December 2021 and December 2022 in *Mangifera indica* plantations located in Universitas Sriwijaya with plantation sizes of 2 ha respectively. The average diameter at breast height (DBH) for the *Mangifera indica* trees was 11 until 15 cm. The surveyed spacing for each plantation was set as 3 × 3

m^2 rows. No fertiliser or fungicide was applied on these plantations.

Disease symptoms that were recorded ranged from canopy appearance (i.e. wilting, yellowing, leaf drying, or defoliation) and black discoloration on the exterior of the trunk, as well as cracks or cankers on the branches and trunk. A variety of observations were also recorded, including the presence of damage by animals, insects, holes from insect attacks, gummosis, and black fluid oozing from wounds on the stem [12];[13]. Other information, such as the planting distance, rotation, source of seedlings, when and if pruning was done, age of seedlings at planting, and fertilizer or pesticide treatments, were also recorded.

2.2 Fungal Isolation

The bark of diseased plants is cut into 1-3 cm lengths from the periphery of the lesion. These wood sections are surface sterilized. The sampled wood was then placed on potato dextrose agar (PDA), and the agar plates were incubated for 10 days at room temperature. Fungi Fungal colonies were subcultured on PDA, and incubated for 10 days. Subcultures of single spores were retained for routine use on tilted PDA. The fungi isolated were initially identified based on the morphological characteristics of the 10-day-old cultures. The sexual and asexual states of the fungi were plugged in lactophenol on glass slides, and examined by microscope Olympus CX23.

2.3 Genomic DNA extraction, PCR amplification, and sequencing

DNA Isolation using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA). For extraction of genomic DNA, the culture was incubated for five days to allow sufficient mycelial growth in potato dextrose broth (PDB) (Merck, Germany). The mycelium was purified with sterile filter paper (Whatman) and transferred to 1.5 mL Eppendorf tubes. The quantity and quality of DNA extracted were evaluated with a spectrophotometer (NanoDrop ND-1000; Thermo Fisher, Waltham, MA, USA) to calibrate the PCR template DNA concentration and purity.

PCR was performed with a C1000 Touch™ thermal cycler (Bio-Rad, USA). PCR cycle protocols were as follows: initial denaturation for 5 minutes at 95°C, followed by 35 cycles at 95°C for 30 seconds, 56°C for 45 seconds, and 72°C for 1 minute. The Amplification was completed at 72°C for 10 min and PCR Products were stored at 10°C [10]. PCR amplifications were made for two gene regions, including part of the b-tubulin (BT) using primers βt1a (TTCCCCCGTCTCCACTTCTTCATG) and βt1b (GACGAGATCGTTCATGTTGAACTC) [14]. The PCR products generated were submitted to 1st BASE (Malaysia). Raw sequence data were manually analyzed and edited using Genestudio 2.1.1.5 (Genestudio, Suwanee, Georgia) and BioEdit software (van der Nest et al. 2019). The DNA sequence was compared to the GenBank database via the BLAST nucleotide-nucleotide search interface located at the National Center for Biotechnology Information, Bethesda, USA. The phylogenetic tree was constructed with MEGA11.

3. Results and Discussion

3.1 Symptoms of *Mangifera indica* wilt disease

We observed symptoms of this disease in a mango plantation in the Sriwijaya University campus area. The disease was found scattered throughout the planted area, with symptoms of partial tree death, leaf wilting or dropping, and tree death (Figure 1. a-b). At initial, the leaves of infected plants reduce turgor and brightness, with symptomatic yellowing of older leaves, followed by wilting and death of the plant. The symptomatic plant stems cancer-forming stems and show xylem discoloration (Figure 1. c-d), the infection generally begins from the roots and then moves up the stem, and finally spread to the upper branches of the whole plant, and the plant finally dies. Death of the nearby plants indicates root transmission as the pathogen is also known as a soil-borne pathogen. Infectious severity is also caused by pruning branches using tools that were previously used to cut infected plants [15].

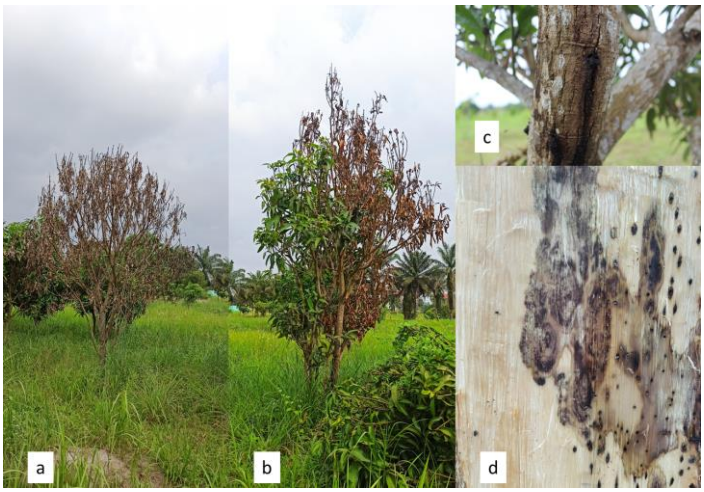

Figure 1. a. a. plants experience wilting and eventually die; b. plants experience partial wilting; c. the formation of cancer on the stems of infected plants; d. on sapwood there are lesions and holes formed by insects.

3.2 Fungal Isolate and morphology

We have successfully isolated five isolates from diseased mango plants. At 5-10 days of incubation at 25 ˚C on MEA medium, the cultures were pale brown to dark brown and produced a banana-like odor. The mycelium on MEA is gray, and the back side of the colony is olive-gray; the submerged mycelium darkens as the ascomata develop, forming fine, radiating fibrils. Ascomata were developed within seven days and matured within ten days, superficially or partially embedded in agar, dark brown to black in color (Figure 2a). Ascomatal bases submerged or on the agar surface, dark bases dark brown to black, subglobose to globose bases. The neck of the ascomata is upright, and occasionally curved, black at the base becoming subhyaline towards the apex, smooth to crenulate, and long including ostiolar hyphae (Figure 2b). Conidiophore/phialide (Figure 2c). Chlamydospores are oblong, thick-walled, and smooth (Figure 2d). Hat-shaped ascospores (Figure 2e). Conidia barrel and conidia are bacillus-shaped (Figure 2f).

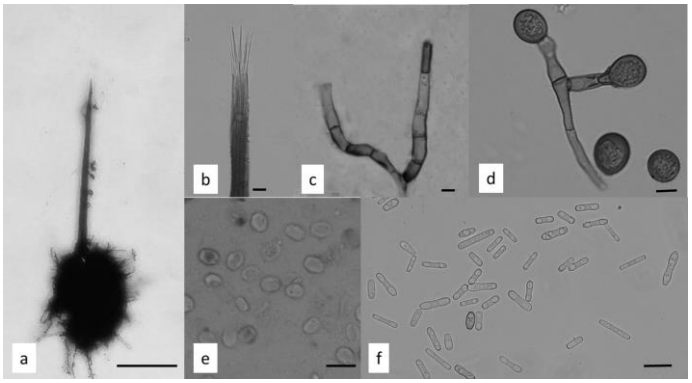

Figure 2. Morphological characteristics of Ceratocystis isolated from *Mangifera indica* stem lesion: a. Globose ascomata with long neck, b. Divergent ostiolar hyphae, c. Conidiophore/phialide, d. Chlamydospores, e. Hat-shaped ascospores. f. Barrel-shaped conidia. Scale bars: a = 100 µm; b, c, d, f = 10 µm; e = 5 µm.

3.3 PCR and sequencing

The β-tubulin region was amplified and sequenced for isolate, with an amplicon size of ± 550 bp in length. Using the β-tubulin gene region, phylogenetic analysis revealed that isolate obtained from wilted *M. indica* trees containing *Ceratocystis fimbriata* from the type collection on *Acacia* sp [16], on *Coffea arabica* [17], on *Eucalyptus grandis* [18], on *Lansium domesticum* [8], *Mangifera indica* [7]; [19] (Fig.3).

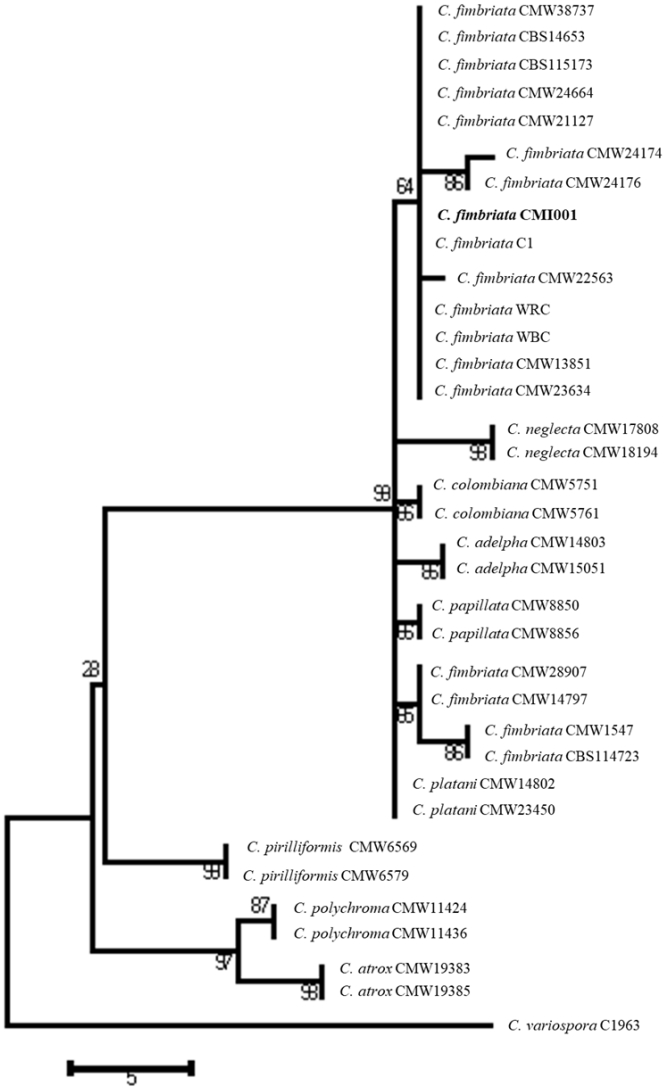

Figure 3. Phylogenetic tree constructed by MEGA with Maximum Parsimony (MP) analysis by a β-tubulin sequences from *Mangifera indica* tree in South Sumatera (marked in bold) and other species in the Latin American and Asian clade of the *C. fimbriata* species complex. The consistency index is (0.933333), the retention index is (0.976190), and the composite index is 0.933123 (0.911111) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

Discussion

This study presents the first report of *C. fimbriata* associated with massive die-back of *Mangifera indica* trees in South Sumatra, Indonesia. The disease caused widespread wood discoloration of the plant's vascular tissue and led to wilting of mango trees found in the field. According to [10];[11], the development of *Ceratocystis* in the vascular tissue of bullet wood and jackfruit plants causes the leaves on the plants to wilt until they dry up and eventually

the plants die. Other symptoms caused by this pathogen are the development of cankers on sapwood and the formation of gum on the tree. Many holes were found in the affected trees caused by the insect *Hypocryphalus mangiferae*. According to [9], this insect was also found on duku plants infected with *Ceratocystis* and became the vector of this disease.

The spread of disease on mangoes is closely related to the wood-boring insect *H. mangiferae* which is known as a vector of Ceratocystis disease in mango plants in Oman and Pakistan [6]; [20], Bullet Wood [11], and *L. domesticum* [9]. *H. mangiferae* are found on diseased stems and form holes, especially in lesions on the wood. Termites are also always seen on infected mango plants and cause wounds on the stem which makes the diseased mango plants become worse and eventually die [8]. In addition, pruning of Ceratocystis-infected branches through the use of unsterilized agricultural tools exacerbates the spread of this disease [11] which is also caused by wind [21]. *Ceratocystis* is also transmitted from infected wild *Acacia* around mango plantations or other plants that host this pathogen [11].

The identity of *Ceratocystis fimbriata* as a pathogen associated with wilt disease in M. indica was determined based on morphological characteristics and DNA sequence comparison of CMI001 with reference isolates CMW38737, C1345, A59662, YM061, P20053, C1, CMW22563, WRC, CMW13851, CMW23634, CMW22579. *C. fimbriata* collected from *M. indica* in South Sumatra is part of the *C. fimbriata* s.l. complex grouped into *C. fimbriata* sensu stricto. A comparison of β-tubulin gene sequences in each isolate obtained showed similarities with *C. fimbriata* reported to attack *L. domesticum* [9], jackfruit [10], and bullet wood [11].

4. Conclusion

This study presents the first report of the disease Ceratocystis wilt on *Mangifera indica* in Indonesia and the detection of the fungus that has been identified as *C. fimbriata*. The mango disease that gave rise to this study is very serious and management choices to reduce its incidence are required *C. fimbriata* is an aggressive pathogen and a deeper comprehension of its possible role in tree die-back will be essential in the future.

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