Spatial distribution and morphological characterization of *Ceratocystis fimbriata* associated with wilt disease of duku in Ogan Ilir, Indonesia

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Abstract. Ceratocystis wilt disease, caused by *Ceratocystis fimbriata*, has emerged as a devastating threat to the sustainability of duku (*Lansium domesticum*) production in South Sumatra. Despite its high severity, the spatial distribution, epidemiological drivers, and characterization of the local pathogen in the Ogan Ilir region remain undocumented. This study aims to describe the spatial distribution of the disease, evaluate the epidemiological dynamics of abiotic and biotic factors, and provide morphological characterization and definitive molecular identification of the causative agent. A field survey at one affected location, followed by aseptic isolation and identification through Internal Transcribed Spacer (ITS) rDNA sequencing, revealed a significant disease incidence, with an incidence rate reaching 73.39% in Miji. Symptoms observed included characteristic xylem discoloration, necrotic stem lesions, and rapid crown decline leading to tree death. Morphological assessment of the isolate was consistent with the genus *Ceratocystis*, and phylogenetic analysis confirmed that the isolate shared >70% sequence similarity with *C. fimbriata*. This study establishes *C. fimbriata* as the primary etiologic agent of lethal wilt disease in *L. domesticum* in Ogan Ilir. Therefore, an integrated management strategy that includes early monitoring, orchard sanitation, and environmental management is crucial to suppress the disease and ensure sustainable *L. domesticum* production.

1 Introduction

Lansium domesticum, commonly known as langsat or duku, is a tropical fruit tree of the Meliaceae family that is widely cultivated in Southeast Asia. This crop has high economic value due to its distinctive organoleptic characteristics, particularly its sweet taste and unique aroma, as well as its content of bioactive compounds in the form of antioxidants that contribute to its health benefits [1]. Duku production in South Sumatra reached 333,649 tonnes, the highest in Indonesia [2]**.** However, the sustainability of duku cultivation is threatened by diseases that can reduce productivity and shorten orchard lifespans. The emergence and spread of destructive diseases in duku orchards could lead to mass extinction in South Sumatra.

Several pathogens have been reported as threats to duku trees in Indonesia and worldwide, causing trunk damage and resulting in a significant drop in production. *C. fimbriata* causes bark canker disease. In severe cases, it can lead to 100% tree mortality within three years in heavily infected orchards [3]. This pathogen has spread throughout all districts in South Sumatra since 2014. Two main haplotypes, ITS5 and ITS7b, are highly pathogenic to duku and several other agroforestry [3]. *Parvodontia austrosinensis*; the mycelium spreads from the upper part of the tree to the crown, causing permanent decay and death [4]. *Fusarium* spp. causes wilt, root rot and canker, which can lead to stunted growth and plant death [5].

During the period 2014–2021, outbreaks of *Ceratocystis* wilt in duku trees were reported across various areas of South Sumatra, indicating an increasingly widespread distribution. Disease symptoms were observed in several districts in South Sumatra, indicating widespread disease spread. A total of 31 affected locations were identified, with the largest number in Ogan Komering Ulu Regency (8 locations**)** [6]. In 2026, cases of duku plant disease were reported in Ogan Ilir Regency, with an infection rate of over 75%; field observations revealed cankers (open wounds) and black lesions on the stems and vascular tissue, similar to the symptoms caused by *C. fimbriata* [7]. The infection causes the leaves to wilt, which can ultimately lead to the death of the entire plant, a discoloration to dark brown or black in the xylem, clearly visible in a cross-section of the stem [8]. *C. fimbriata* has a wide host range and has been reported on woody plants of high economic value, including acacia, eucalyptus and pequi [9], [10], [11]. This pathogen begins by infecting wounds on the stem, allowing the spores to colonize the vascular tissue. The spread of the pathogen is facilitated by bark beetles, which bore galleries into the stem, thereby spreading the spores from healthy plants to diseased ones [12]. These characteristics make *Ceratocystis* one of the most important and destructive

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vascular pathogens in tropical and subtropical ecosystems.

Although this disease has been reported in South Sumatra, comprehensive information on its spatial distribution, the dynamics of abiotic and biotic factors, and the characterization of the causative pathogen in the Ogan Ilir region has not yet been reported. It is important to address this knowledge gap given its implications for disease control strategies and the sustainability of duku production. This study aims to determine the distribution of duku disease in Ogan Ilir, South Sumatra; the dynamics of abiotic and biotic factors influencing disease distribution; the morphological and microscopic characteristics of isolates; and species identification based on genetic marker analysis.

2 Materials and methods

2.1 Disease distribution survey and sample collection

A field survey was conducted in January 2026 in a duku agroforestry landscape in Ogan Ilir Regency, Indonesia. Observations were carried out in Kandis Sub-district (Fig. 1). This survey used a purposive sampling method, with locations selected based on the main production centers of *L. domesticum* in Ogan Ilir Regency. Observations were conducted using a census method, determining the percentage and severity of the disease [13].

Disease severity was assessed using a scale of 0–4 (Table 1) [13], taking into account both leaf canopy wilting and the presence of diagnostic stem lesions. To minimise misclassification arising from non-pathogenic factors such as drought or root rot, a tree was only recorded as infected if the wilted leaves were accompanied by symptoms characteristic of *Ceratocystis*, specifically a linear change in the colour of the sapwood from brown to black or cracked bark.

$$I = \frac{n}{N} \times 100\%$$

where;
I = disease incidence
n = number of infected plants
N = total number of observed plants

$$\text{Disease severity Index (DSI)} = \frac{\sum(nxv)}{NxV} x100\%$$

where;
S = disease severity (%)
n = number of plants (or leaves) in each disease severity class
v = disease severity score for each class
N = total number of plants (or leaves) assessed
V = maximum disease severity score

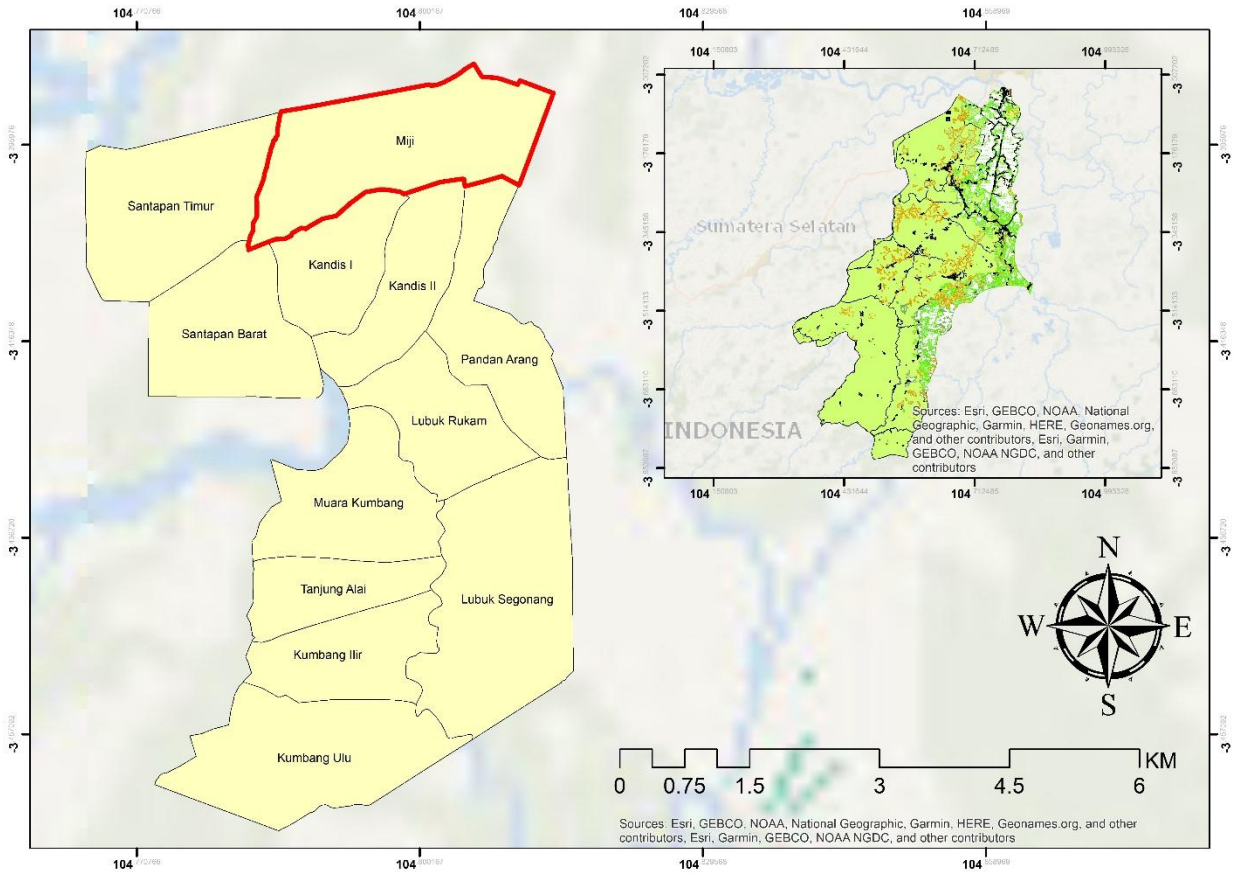

Fig. 1. Map showing the research location in Ogan Ilir Regency, South Sumatra

Table 1. Scoring of disease severity in duku plants

Score	Description
0	A healthy tree with no wilted leaves or visible lesions
1	1–25% of the leaves are wilted, usually associated with localised lesions on small branches
2	25.1–50% of the leaves are wilted, with lesions spreading towards the main stem
3	50.1–75% of the leaves are wilted, often accompanied by extensive bark cracking and deep discolouration of the sapwood throughout the trunk
4	75.1–100% of the leaves are `wilted or have fallen off entirely, indicating systemic colonisation of all vascular tissues

A duku tree is classified as infected if its branches or trunk exhibit characteristic symptoms, such as leaf wilting accompanied by lesions on the trunk or branches and a discolouration of the sapwood to brown or black. From each affected orchard, five symptomatic *L. domesticum* trees were randomly selected for the collection of symptomatic sapwood samples. Tissue samples were taken from the sapwood showing discolouration by peeling the bark and cutting the infected xylem longitudinally to a length of approximately 50 mm. The samples obtained were then wrapped in sterile tissue, placed in labelled plastic bags, and stored at 4°C before isolation in the laboratory.

2.2 Isolation, identification and morphological characterisation

Ceratocystis was isolated using two methods: the first involved cutting a 20 × 20 mm sample from diseased wood. The wood sample was sterilised by washing it in sterile distilled water for 5 minutes, then placing it in a streptomycin solution for 5 minutes, followed by sodium hypochlorite (NaClO) for 5 minutes, and rinsing with sterile distilled water. Afterwards, the wood sample is dried in a laminar airflow and inoculated directly onto malt extract agar (MEA) at room temperature (25°C) for 7–10 days to induce direct sporulation on the MEA [14]. The second method uses the carrot bait technique. Samples of diseased wood of the same size as described previously were placed between slices of fresh carrot and incubated in a humid chamber at room temperature for six to eight days. The single ascospore masses developing at the tips of the ascomata on the wood and carrot slices were transferred to 2% MEA (20 g/l malt, 20 g/l agar) (Biolab, Midrand, South Africa) in new Petri dishes, after which these cultures were incubated for four to six days at 25°C

Isolates were selected to represent the survey area of Kandis Sub-district (SS-DD). Macroscopic observations were then systematically carried out to evaluate colony characteristics, pigmentation, and growth rate. Observations were conducted using an Olympus CX33 microscope equipped with Optilab Advance Plus. To ensure high morphological accuracy and to capture a comprehensive range of structural variability, 100 random observations were recorded for each isolate regarding the length and width of the ascomata (base and neck), ascospores, basiform conidia, barrel-shaped conidia, and chlamydospores,

2.3 DNA extraction, amplification, sequencing and phylogenetic analysis

Single-spore cultures were prepared from fungal isolates representative of each region infected with *Ceratocystis*. The isolates were cultured in Potato Dextrose Broth (PDB) in 250 mL Erlenmeyer flasks at 25°C for 10 days. The mycelium from the PDB cultures was filtered, transferred to tubes, and crushed with a Micropestle until a fine powder was obtained. Genomic DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA**)**. DNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA). Polymerase Chain Reaction (PCR) amplification and sequencing were performed for the internal transcribed spacer (ITS) region, comprising: ITS1 (TC CGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCT TATTGATATGC) [14]. Amplification was carried out in a 50 µl reaction containing 20 µl Master Mix (Eppendorf, Hamburg, Germany) (25 mM $MgCl_2$, 0.06 U/µl Taq DNA polymerase, 0.2 mM of each dNTP), 1 µl each of forward and reverse primers, 1 µl template DNA, and 27 µl sterile water. PCR was performed using a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The parameters were an initial denaturation for 3 minutes at 94°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C. Amplification was completed at 72°C for 10 minutes, and the PCR products were stored at 10°C. Visualisation was performed using a Gel Doc XR+ (Bio-Rad, Hercules, CA, USA) to assess the quality and presence of the amplified products.

The verified PCR products were then sent to 1st BASE (Malaysia) for Sanger DNA sequencing using the same set of primers previously mentioned. DNA sequences from several *Ceratocystis* spp. selected from previous studies (Table 2) were compared with the DNA sequences sequenced in the current study using the GenBank database via a BLAST nucleotide search located at the National Center for Biotechnology Information (NCBI), Bethesda, MD, United States. Matching sequences were transferred and subsequently processed using BioEdit software (Hall, 1999). The tree was visualised and edited in MEGA v. 11 using maximum likelihood analysis and 1,000 bootstrap repetitions [8]. Branch support for nodes was obtained by performing 1,000 bootstrap repetitions of the aligned sequences. For the maximum likelihood analysis, the

metrics calculated included tree length, retention index and consistency index.

Table 2. *Ceratocystis* isolates included in the phylogenetic analysis based on the internal transcribed spacer (ITS) region

Isolate	Accession Number	Species	Host	Country
WRC	MT229127	*C. fimbriata ITS5*	*L. domesticum*	Indonesia
YM061	AM712445	*C. fimbriata ITS5*	*Colocasia esculenta*	China
C2055	HQ157548	*C. fimbriata ITS6*	*Mangifera sp.*	Brazil
WBC	MT229128	*C. fimbriata* ITS6z	*L. domesticum*	Indonesia
P20053	AM292204	*C. fimbriata* ITS5	*Punica granatum*	China
A59662	KF650948	*C. fimbriata*	*Camellia sinensis*	China
SS-DD	PZ438006	*C.fimbriata* ITS5	*L. domesticum*	Indonesia (present study)

3 Results and discussion

3.1 Distribution and symptoms of *C. fimbriata* attacks

Trees infected with *C. fimbriata* exhibit leaf wilting, with the leaves turning orange-brown and eventually falling off (leaving the plant bare) (Fig. 2A–B). Wilted stems display brown to black streaks in the xylem. Symptoms on duku wood produce grey to brown lesions, including a pattern of discoloured streaks in the sapwood (Fig. 2C) and, in some cases, lesions extending into the heartwood (Fig. 2D). Lesions may be found affecting the sapwood partially or entirely, from the base of the stem to the branches. Leaves on dying trees exhibit yellowing, followed by leaf wilting on some lateral branches, twig desiccation, and the wilting of the entire tree. This disease has been observed in Kandis and Sungai Pinang, Ogan Ilir, South Sumatra. Affected trees are typically mature (>40 years) with a disease incidence of >75% (Table 2). Based on observations of the lesion areas, holes were found that serve as entry points for ambrosia beetles, which act as vectors for the transmission of *C. fimbriata* spores (Fig. 3).

Fig. 2. Symptoms of *Ceratocystis* wilt on a duku tree (*Lansium domesticum var. domesticum*). (A) Total dieback of the upper branches of the plant. (B) Trees infected with Ceratocystis fimbriata exhibit rapid and simultaneous leaf wilting on the main branches or across the entire canopy, eventually leading to death. (C) Black lesions on the stem (D) Discolouration of the wood extending to the heartwood of the lower stem

Fig. 3. Ambrosia beetles

Table 3. Incidence of wilt and severity of *Ceratocystis* disease in Duku orchards in Ogan Ilir District

Location	Number of observed plants	Number of diseased plants	Disease incidence (%)	Disease severity
Kecamatan Kandis (Desa Miji)	126	95	73.39	56.00

3.2 Microscopic characteristics

The morphological characteristics of the reproductive structures were observed using an Olympus CX33 optical microscope. The ascospores have a dark brown to black base, with a subglobose to globose shape, measuring (n = 100) 56.43–223.30 × 49.51–245.84 µm (Fig. 4A). The neck of the ascoma appears erect, sometimes slightly curved, black at the base and becoming subhyaline towards the tip, with a smooth to slightly serrated surface, measuring 90.90–531.74 × 6.77–25.69 µm (Fig. 4B). Barrel-shaped conidia measure 6.05–18.88 × 4.20–8.36 µm in length (Fig. 4C). Ostiolar hyphae measure 11.45 × 87.09 µm in length. Chlamydospores measure 8.43–11.68 × 9.33–14.65 µm (Fig. 4D). Cylindrical conidia measure 10.04–27.39 × 3.65–7.48 µm (Fig. 4E). Cap spores measure 2.82–6.41 × 2.24–5.11 µm (Fig. 4F). Overall, the observed morphological characteristics of the reproductive structures were consistent with those of *C. fimbriata.*

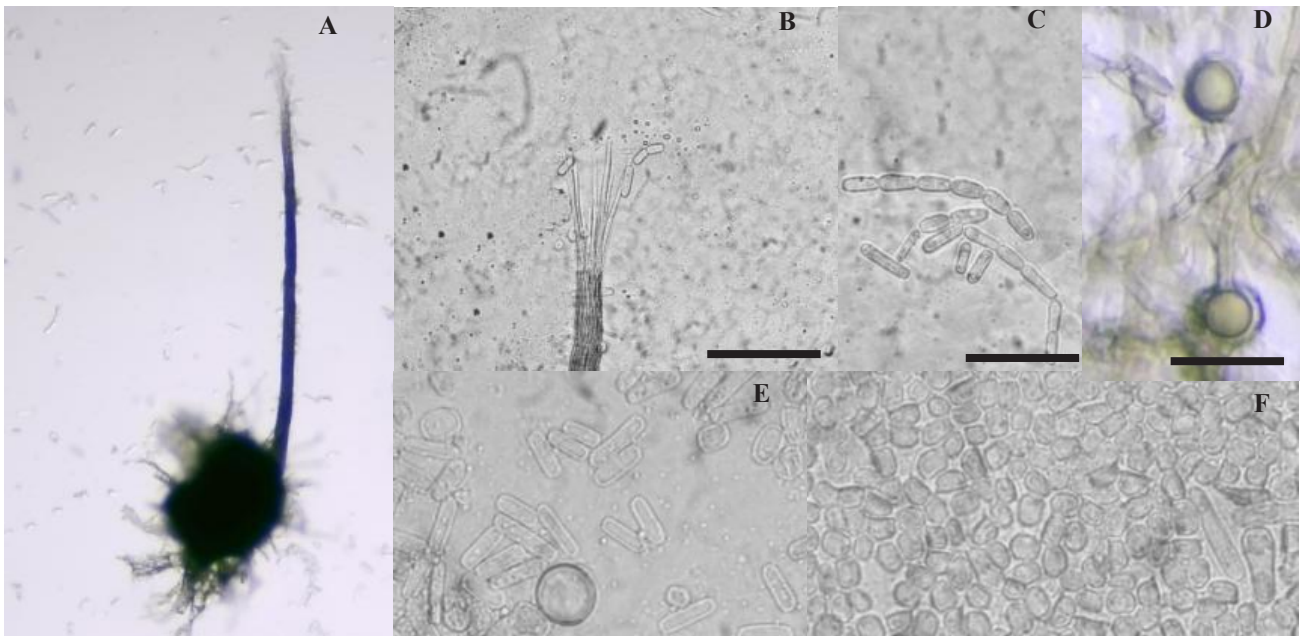

Fig. 4. Morphological characteristics of *Ceratocystis fimbriata* isolate SS-DD from bark canker of *Lansium domesticum*. (A) ascomata with pirilliform base (B) divergent ostiolar hyphae; (C) barel shaped conidia (D) chlamydospore; (E) Cylindrical conidia (F) hat-shaped ascospores. Scale bars = 100 µm (A), 10 µm (D), 50 µm (B,C-F).

3.3 Phylogenetic analysis

Phylogenetic analysis based on ITS sequences showed that the isolates obtained from SS–DD clustered in the *C. fimbriata* clade along with reference isolates retrieved from GenBank. This clade was supported by a bootstrap value of 71%, indicating moderate statistical support. Meanwhile, *C. virescens* was positioned separately as an outgroup. These results confirmed that the isolates used in this study belonged to *Ceratocystis fimbriata* (Fig. 5).

Fig. 5. Phylogenetic tree based on ITS gene sequence data. Isolates in bold were isolated from Indonesian duku and sequenced as part of this study. Phylogenetic analysis used the Maximum Likelihood method. *C. virescens* is the outgroup

3.4 Discussion

Between 2025 and 2026, duku wilt cases were reported in Ogan Ilir Regency, South Sumatra, in the village of Miji (73.39%). Symptoms of the disease observed in the field included wilting of the plant canopy, followed by a change in leaf colour to yellowish-brown, necrosis, progressive leaf drop, and eventually the complete death of the plant. On the stems, cracks in the bark, black lesions, and discolouration of the xylem tissue to brown or black were found. These symptoms of sudden death are similar to those caused by *C. fimbriata*, which infects *L. domesticum* in OKU [6] and *A. mangium* [9].

Morphological characterisation indicates that all isolates exhibit the characteristics of *C. fimbriata*, including dark–coloured ascomata with elongated necks, divergent ostiolar hyphae, barrel-shaped conidia, cylindrical conidia, chlamydospores, and cap-shaped ascospores. These structures are consistent with the morphological description of *C. fimbriata* previously reported on *Eucalyptus* and *Metrosideros polymorpha* [10], [15] Based on a phylogenetic analysis using MEGA11 (Maximum Likelihood), the SS-DD isolate belongs to the species *C. fimbriata*, with a similarity of over 70% to the WBC and WRC isolates that infect *L. domesticum* in Ogan Komering Ulu [3]. The spread of *Ceratocystis* among host plants is closely associated with ambrosia beetles (Coleoptera: Curculionidae), particularly species of the genera *Xylosandrus*, *Xyleborus*, and *Euplatypus*. These beetles have been reported on a wide variety of host plants, including mango (*Mangifera indica*), cacao (*Theobroma cacao), Acacia* spp., and *Eucalyptus* spp., and have been associated with several *Ceratocystis* species, including *C. fimbriata*, *C. manginecans*, and *C. cacaofunesta*. *Ceratocystis* spores have been isolated from the beetles' body surfaces, and transmission experiments have confirmed that some ambrosia beetles can transmit the pathogen to healthy plants, primarily through fresh wounds. Additionally, *Ceratocystis* DNA has been detected in beetle feces, further reinforcing their role in the spread of the pathogen [12]. In this study, ambrosia beetles were found in hollowed-out duku fruits, acting as vectors for the spread of spores. Furthermore, the disease was found to affect all age groups of the plants, ranging from young plants under 10 years old to mature plants over 75 years old. These findings indicate that *C. fimbriata* possesses a high infectivity rate that is not significantly influenced by the age of the host plant. The pathogen's ability to infect young plants suggests that plant tissues still actively growing remain susceptible to infection by vascular pathogens.

4 Conclusion

Based on a field survey, *Ceratocystis* wilt in duku trees in Ogan Ilir Regency, South Sumatra, shows high disease incidence and severity, particularly in older trees within agroforestry areas. The main symptoms observed include leaf wilting, discolouration of xylem tissue to brown or black, the formation of lesions on the stem, and the death of the canopy or the entire plant. Isolation and morphological characterisation indicate that the isolates obtained possess macroscopic and microscopic characteristics consistent with *C. fimbriata*, including the characteristic forms of ascospores, conidia, and ascospores. Phylogenetic analysis based on the ITS1 and ITS4 gene markers confirmed that the SS–DD isolate shares >71% sequence similarity with *C. fimbriata* entries in GenBank. These findings indicate that *C. fimbriata* is the primary pathogen causing wilt disease in duku in the Ogan Ilir region and has the potential to pose a serious threat to the sustainability of the duku agroforestry system in South Sumatra.

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