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Isolation and characterisation of pathogenic and non-pathogenic fungi associated with avocado plants showing dieback symptoms in Indonesia

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Abstract: The avocado (*Persea americana* Mill.) is a high value fruit crop in Indonesia. This exotic commodity is affected by dieback disease, an unrecorded disease in the country that threatens the production. The objectives of the present study were to characterise the pathogen and culturable non-pathogenic fungi associated with the dieback disease of avocado plants. Fungal isolates were collected from branches of avocados showing dieback symptom in the Standard and Instrument Tropical Fruit Applied Institute (SITFAI) experimental orchards during 2022–2023. A total of 17 fungal isolates selected from 73 fungal isolates isolated from three location were characterised morphologically, molecularly, phylogenetically, and by pathogenicity tests. The fungal isolates were tested for their pathogenicity to the local variety of avocado with two stages and three replications. The identification of the fungal species was conducted on the morphological characteristics and molecular analysis obtained from the internal transcribed spacer (ITS), the 28S region of the ribosomal DNA, and translation elongation factor 1 (TEF1). The results revealed that the artificial inoculation of Avo7 and Avo3.2 isolates, identified as *Lasiodiplodia theobromae*, caused necrosis and wilt symptoms on the avocado seedlings. Several fungal species from the Botryosphaeriaceae, Eurotiomycetes, and Sordariomycetes groups were found alongside the pathogen responsible for causing the dieback symptoms in the avocados. The most frequently isolated genera were fast growing, Botryosphaeriaceae (58.9%), followed by *Penicillium* spp. (20.5%), *Pestalotiopsis* spp. (15.1%) and *Colletotrichum* spp. (5.4%). The information in this article should be used as new insights about the incidence of dieback disease caused by *L. theobromae* and proper management strategies against dieback disease on avocado need to be developed.

Keywords: *Persea americana* Mill.; *Lasiodiplodia theobromae*; avocado dieback; fungal disease

The avocado (*Persea americana* Mill.), from the *Lauraceae* family, grows in subtropical and tropical areas worldwide and is native to the Central America, Mexico and Guam regions. The exotic commodity was introduced into Indonesia by the Spanish in the 18th century (Silva et al. 2014). Now-

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adays, more than 20 varieties exist in Indonesia and they have become an important commodity (Dastama et al. 2022). The total production in the country reached 175.7 t in 2020 and the largest producer is the East Java region (BPS 2021). The avocado has many health benefits. The nutrients and phytochemicals in the avocado fruit can control blood pressure, selectively inhibit cancer cells, and can be used as a treatment for cardiovascular diseases and diabetes (Bhuyan et al 2019). The avocado contains antioxidants, such as zeaxanthin and lutein, that maintain one's eye health (Lu et al. 2005; Bhuyan et al. 2019).

Several fungal taxa have been confirmed to affect avocado plants with varying symptoms (Perez-Jimenez 2008). One of them, *Phytophthora cinnamomi,* is considered the most destructive and widely spread pathogen that causes avocado decay. In recent decades, the research focus on perennial crops, including avocados, has turned to the diseases caused by latent pathogens whose manifestations include shoots and twigs to decay and dieback. The damage called dieback disease is mainly caused by the Botryosphaeriaceae species (Rodríguez-Gálvez et al. 2021). The Botryosphaeriaceae family is also known to act as a latent opportunistic pathogen. When the host is exposed to stressful conditions, it becomes pathogenic (Slippers & Wingfield 2007). There have been many reports of infections by this group of pathogens in avocado-producing countries and the resulting losses are considerable (Zea-Bonilla et al. 2007; Ramírez-Gil & Morales-Osorio 2021; Liang et al. 2021; Fiorenza et al. 2022). *Neofusicoccum parvum* is the main cause of dieback disease in avocados in Spain and Mexico (Zea-Bonilla et al. 2007; Ramírez-Gil & Morales-Osorio 2021) and losses are increasing year on year. In Colombia, dieback is caused by *Lasiodiplodia theobromae* among other pathogens (Ramírez-Gil & Morales-Osorio 2021). In Taiwan, dieback is caused by *L. theobromae*, and *L. pseudotheobromae* (Liang et al. 2021). In Italy, the recently identified causal pathogens are *N. siciliana* and *N. rosae* (Fiorenza et al. 2022).

Apart from the pathogen, a group of various fungi can colonise the internal tissues of plants without causing symptoms. They are key components of plant symbiosis, influencing the host's tolerance to environmental stresses (Azad & Kaminskyi 2015), plant defence (Tiwari & Singh 2021) and plant growth (Tkacz & Poole 2015). Their interaction can be mutualism, commensalism, latent pathogenicity, and saprophytism. Non-pathogens are found to have antagonistic properties and can be candidate biocontrol agents. The aim of this study was to characterise the pathogenic fungal and culturable non-pathogenic species isolated from typical symptoms of dieback disease on the avocado and to test their pathogenicity on the host plant.

MATERIAL AND METHODS

Sources of the material and fungal isolation. Several trees showing dieback disease were observed on ten experimental orchards and backyards of the Standard and Instrument Tropical Fruit Applied Institute (SITFAI), located in the X Koto Singkarak Subdistrict, Solok district West Sumatera. In 2022, two sites located in the Sumani orchards were evaluated for the presence of dieback symptoms. The sites are located at 0°43'11.81" S, 100°35'54.94" E, and 0°43'11.81" S, 100°35'54.94" E. The sampled cultivars were *P. americana.* Mill. cv. Mega Murapi, Mega Paninggahan and Mega Gagauan. In each location, two trees were selected, and five branches from each tree were sampled as the replicates. The avocado cultivars were cut in 5–10 cm long symptomatic branches. A total of 20 samples were collected for the fungal isolation. After peeling off the bark, the branches were sterilised with 96% alcohol for 3 min, washed twice with sterilised water before isolating the fungi in fresh Potato Dextrose Agar (PDA, Merck, Germany) and PDA acid media. The cultures with wood pieces were then incubated at 25–30 °C for 7 days. The growing fungal colonies were re-cultured to fresh PDA and single spore cultures were obtained as described by Hilário et al. (2020).

Pathogenicity test of the isolates. Twelve selected fungal isolates were tested for their pathogenicity conducted through the mechanical inoculation of the 'local' avocado rootstock plants. For each isolate, three potted 1-year-old healthy seedlings (1 cm in stem diameter and 15 cm in height) were used for the inoculation. Before inoculation, the stem surface was disinfected with 75% ethanol and wiped with a sterile tissue. Stem barks were slashed 0.05 cm in depth and 1 cm in length using a sterile scalpel blade. Each plant received three incisions. Subsequently, a 0.5 cm^2 mycelial plug from a 15-day-old culture

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was inoculated into the inner side of the incision and wrapped with Parafilm® (American National Can, USA) to prevent desiccation. The mycelial plug was placed at a distance of 5 cm between the incisions and 20 cm from the basal seedling. An equal number of plants were inoculated with sterile PDA plugs to serve as the control. All the inoculated plants were kept in a 70% shade house (ambient temperature 25–30 °C) and maintained for a month. During that period, the plants were monitored weekly for the development of symptoms and the dimensions of the necrosis were measured after 1 month. Four isolates that induced necrosis on the avocado stem were re-confirmed for their pathogenicity. In the new pathogenicity assay, each isolate was inoculated into three plants and each plant with three slashed points in the stem were used as the replication.

DNA extraction, Polymerase Chain Reaction (PCR), and phylogenetic tree analysis The genomic DNA was isolated from 0.2 g of the 7-day-old fungal isolates using a Quick-DNA Fungal/Bacterial Microprep Kit (Zymo Research, Irvine, USA) according to the manufacturer's instructions. The isolated fungal DNA was measured for the quantity and quality using a BioSpectrometer (Basic, Germany) at absorbance ratio A260/280 nm.

Molecular identification was carried out by polymerase chain reaction (PCR) amplification and sequencing of the ribosomal DNA ITS1-5.8S-

Figure 1. Symptoms of leaf wilt and brown necrosis that occur around the 1 cm^2 slices of avocado seedling stems mechanically inoculated with 1 cm^2 of the Avo3.2 isolate culture (A) and Avo7 isolate culture (B), necrosis symptom on the seedling inoculated with Avo2 the isolate (C), less symptoms in the avocado stem seedling covered with a clear PDA medium (D) at one month after inoculation, and an area of necrosis measured on the inoculated 12-month-old local avocado cv. seedling with four selected isolates incubated for one month by manual inoculation. Mean necrosis area (mm) caused by Avo2, Avo3.2, Avo5 and Avo7 in the local avocado incubated for one month at 25–30 °C after inoculation. Columns represent the mean, the error bars show positive standard deviation. Mean of the necrosis length values followed by the different letters are significantly different according to Tukey's test (P = 0.05)

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Figure 2. Branch dieback symptoms on an avocado (*Persea americana* Mill.) tree (A, B); Basipetal necrosis of the stem with necrosis of the internal tissues (C)

ITS4 region (ITS1; 5'-TCCGTAGGTGAACCTGCGG -3', ITS4; 5' CCT CCGCTTATTGAT-3') using a thermocycler (Eppendorf® Mastercycler® Nexus Thermal Cyclers, Hamburg, Germany) and the large subunit (LSU) ribosomal (LSU pn2; 5'-GTTCACCATCTTTCGGGT CC-3', LSU pn9; 5'-CTTAAGCATATCAATAAGCGGAGG-3') and translation elongation factor EF-1 alpha (EF1-728F; 5'-CATCGAGAAGTTCGAGAAGG-3', EF1-986R; 5'-TACTTGAAGGAACCCTTACC-3') using a thermocycler (Eppendorf® Mastercycler® Nexus Thermal Cyclers, Germany). For the Internal Transcribed Spacer (ITS) primers pair, the genomic DNA was amplified using a Kappa taq PCR kit (Sigma Aldrich, United States) which had denaturation at 94 °C for 1 min; 28 cycles of denaturation for 15 s at 95 °C; primer annealing at 50 °C for 15 s and extension for 5 s at 72 °C and a final extension at 72 °C for 10 min. For the primers set to the target LSU region and TEF1-alpha region, the genomic DNA was synthesised using the Promega GoTaq® Green Master Mix (Promega, USA) with a thermal cycler (Bio-Rad C1000TM, Hercules, USA) with denaturation at 95 °C for 3 min; 35 cycles of denaturation for 30 s at 95 °C; primer annealing at 55 °C for 30 s and extension for 30 s at 72 °C and a final extension at 72 °C for 5 min.

The PCR products were visualised on 1.2% agarose gel using electrophoresis at 100 V for 30 min. The PCR products were purified and sequenced by The National Research and Innovation Agency (NRIA) Genomic laboratory (Cibinong, West Java). The nucleotide sequences were aligned using Bioedit software and paired with the reference gene using Emboss Needle and the aligned nucleotides were compared to the related species using the BLAST algorithm with GenBank database (Altschul et al. 1990). The phylogenetic relationship among the isolates and reference gene was constructed using Neighbour Joining methods implemented in the MEGA software (version 11), program (Tamura et al. 2021) and bootstrapped with 1 000 replicates.

Statistical analysis. The means and standard deviations of three replicates of the data shown in Figure 1 were examined for differences using Minitab ver. 19 (Minitab, State College, USA) and an analysis of variance (ANOVA). Tukey's HSD test was used to further assess the differences for significance at $P < 0.05$.

RESULTS

Characterisation of the symptoms on avocado plants. Of the ten plantations observed, four plants were taken as samples. The symptoms in three of the observed plants manifested as defoliated branches and then all of the leaves fell off. The basipetal of branch showed necrosis and the stele tissue looked brownish (Figure 2).

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Figure 3. The pathogenicity test results on the avocado seedling screening with 12 isolates

Pathogenicity test. Of the 73 fungal isolates isolated from the stem branch with typical dieback symptoms on three avocado cultivars in the PDA and PDA acid medium. The visual morphology comparisons of the fungal colonies with the available reference isolates characterised the 4, 11, 15, and 43 isolates as *Colletotrichum* sp., *Pestalotiopsis* sp., *Penicillium* sp., and unknown1, respectively. Twelve different isolates labelled as Avo1, Avo2, Avo2.1, Avo3.1, Avo3.2, Avo5Avo7, Avo8, Avo12, Avo13, Avo14 and Avo15 were selected and evaluated for their pathogenicity. All the isolates caused mild to dark brown necrosis only on the wounded area of the local avocado cultivar, except for the Avo7 isolate and the Avo3.2 isolate (Figure 3). The pathogens Avo3.2 isolate caused wide necrotic, and Avo7 caused wilting on the young avocado leaves and brown necrosis on the seedling stems.

Two isolates (Avo7 and Avo3.2) which showed wilt symptoms in a previous test along with two other 2 isolates (Avo2 and Avo5) were evaluated and found to have varying effects on the avocado stems. The average area of necrosis between the isolates is shown in Figure 1. Isolate Avo7 causes the widest necrosis symptoms on the avocado stems, the length of the stem lesion reached 140.92 mm with a value that is statistically significantly different from the length of the lesions by the other isolates.

Isolate Avo3.2 can cause necrosis symptoms with a narrower necrosis area than by isolate Avo7, which had an average of 48.66 mm. The two isolates, Avo2 and Avo5, caused the lowest necrosis of 9.16 and 8.166 mm, respectively, and is thought that the symptoms were localised lesions, and the infection did not develop after attachment of the inoculum source to the stem slices until one month. To meet the requirements of Koch's postulates, the Avo3.2, and Avo7 isolates were re-cultured from the inoculated avocado stem to the PDA medium and were then re-identified.

Morphological characterisations of the fungal collections. A total of 17 isolates, four isolates (Avo2, Avo3.2, Avo5 and Avo7) evaluated in the pathogenicity test and the other isolates isolated from the avocado stem, were described for their morphological characteristics such as the colony colour and conidial shape (Figures 4 and 5).

The Avo7 and Avo3.2 isolates, which cause wilt symptoms on avocado leaves, form rapidly growing colonies. Initially, the colonies are white and resemble cotton, later turning greenish-grey and eventually becoming dark greyish as they com-

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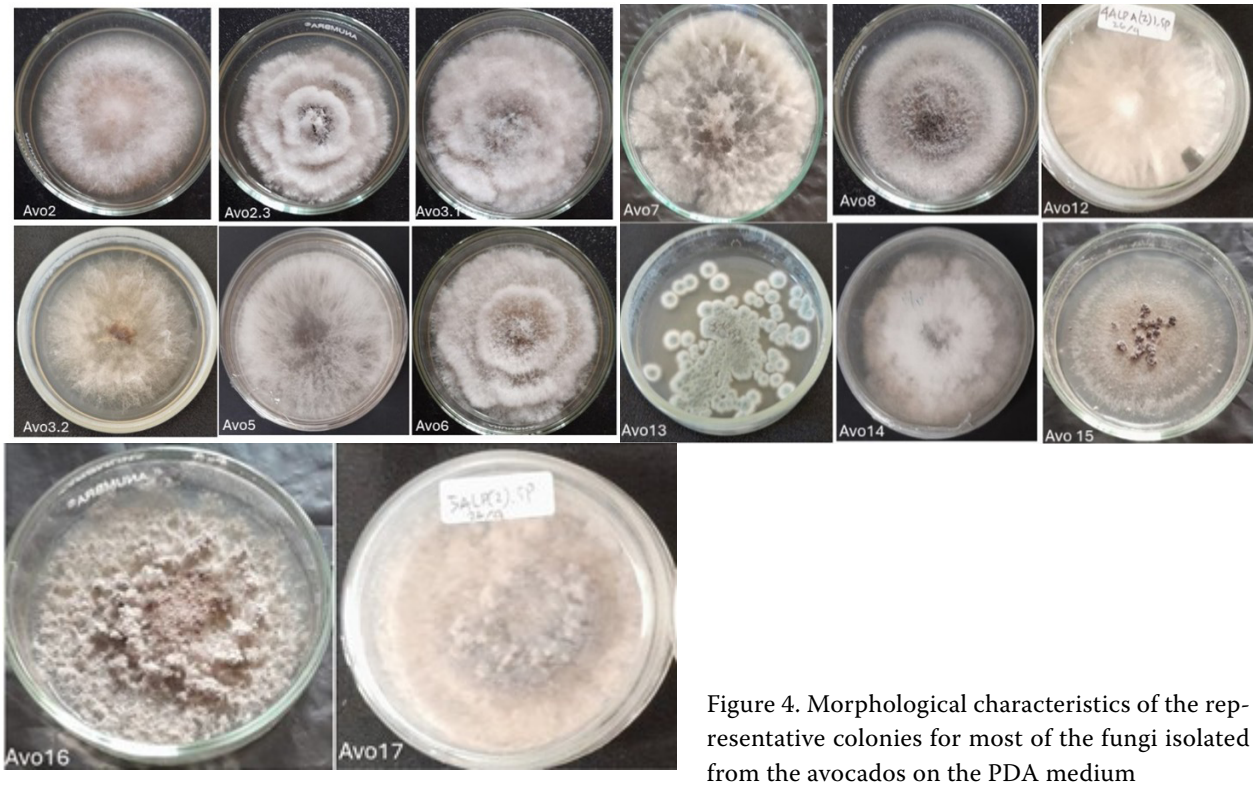

Figure 4. Morphological characteristics of the representative colonies for most of the fungi isolated from the avocados on the PDA medium

pletely cover the petri dish. In contrast, four other isolates (Avo4, Avo5, Avo14, and Avo16), which are indistinguishable from the Avo7 isolate based on their mycelia, did not produce wilt symptoms in the pathogenicity tests. The Avo3.1 and Avo6 isolates that were identified had a white cottony fine texture, had a thick circular mycelium pattern on the PDA which is not that different to the Avo2.3 isolate visually (Figure 4).

A total of four isolates, designated as Avo1, Avo2, Avo8 and Avo15, are typical of *Colletotrichum* isolates. The morphology of *Colletotrichum* was characterised by a dense white mycelium at the beginning to a grey-green mycelium. Some isolates showed the presence of orange-coloured spore masses that were produced in the culture (Figure 4). One selected fungal (Avo13) isolate of 14 isolates with the same characteristics is from the genus *Penicillium* sp. (Figures 4 and 5). The macroscopic characteristics of this group are colonies with grey to dark green with yellow on the edges, and the surface is rough. The Avo2.1 isolate has a colony that is initially white then turned greyish brown and the Avo12 isolate that had a circular, white, smooth, and generative hyphae and had a packed together mycelia type on the PDA (Figure 4). The other fungal Avo17 isolate had a circular, white to light grey dense colony at the margin and a light to dark brown colony at the centre (Figure 4).

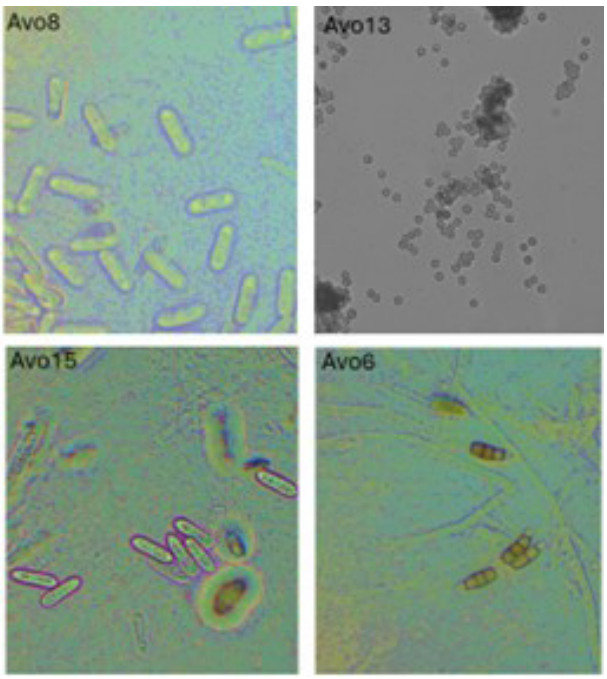

Figure 5. The form of conidia/conidiogenous cell of four representative isolates (Avo8, Avo13, Avo15, and Avo6 isolates grown in a PDA medium)

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Table 1. The molecular characterisations of the branch-associated fungal and pathogens isolated from three avocado cultivars showing dieback disease

Fungal code	Species	NCBI accession No.	Reference sequence accession No.	Identities between nucleotide (%)
Internal Transcribed Spacer (ITS)				
Pathogen				
Avo3_2	*Lasiodiplodia theobromae*	LC782331	MT644474	100.00
Avo7	N/A	–	–	–
Non pathogen				
Avo1	*Colletotrichum fruticola*	LC782306	OR327638	99.10
Avo2	N/A	–	–	–
Avo2_1	*Nigrospora sphaerica*	LC782328	MN337134	100.00
Avo2_3	*Neopestalotiopsis* sp.	LC782329	MK050621	100.00
Avo3_1	*Pestalotiopsis* sp.	LC782330	KT342878	100.00
Avo4	*Pseudofusiccoccum ardesiacum*	LC782332	MF281194	100.00
Avo5	*Lasiodiplodia* sp.	LC782333	MW775466	99.10
Avo6	*Neopestalotiopsis* sp.	LC782334	MK050621	100.00
Avo8	N/A	–	–	–
Avo12	N/A	–	–	–
Avo13	*Penicillium* sp.	LC782335	MT606202	100.00
Avo14	*Neofusicoccum parvum*	LC782336	MN856242	100.00
Avo15	N/A	–	–	–
Avo16	*P. ardesiacum*	LC782337	MF281194	100.00
Avo17	N/A	–	–	–
Large subunit (LSU)				
Pathogen				
Avo3.2	*L. theobromae*	LC787281	OK056572	97.60
Avo7	*L. krabiensis*	LC787285	MN017859	99.85
Non pathogen				
Avo1	*C. fruticola*	LC787279	MH877073	99.85
Avo2	*Colletotrichum* sp.	LC787276	MK411296	99.85
Avo2.1	*N. sphaerica*	LC787280	KY806266	98.86
Avo2.3.	*Neopestalotiopsis* sp.	LC787276	MN860104	98.79
Avo3.1	*Pestalotiopsis* sp.	LC787278	MN181370	99.85
Avo4	*P. ardesiacum*	LC787282	NG_069902	98.67
Avo5	*Lasiodiplodia* sp.	LC787283	MG321678	98.86
Avo6	*Neopestalotipsis* sp.	LC787284	OL657048	99.85
Avo8	*C. tropicale*	LC787286	MH877138	99.30
Avo12	*Nemania bipapilata*	LC787287	MN611220	100.00
Avo13	*Penicillium citrinum*	LC787288	MH665234	99.85
Avo14	*N. parvum*	LC787289	MT183498	99.01
Avo15	*C. tropicale*	LC787290	MH877138	99.70
Avo16	*P. ardeasiacum*	LC787291	NG_069902	99.57
Avo17	*Neoroussoella leucaenae*	LC787292	NG_070073	99.57
Trans elongation factor alpha 1 (Tef 1)				
Pathogen				
Avo3.2	*L. theobromae*	LC790043	MN114216	97.81

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Table 1. To be continued...

Fungal code	Species	NCBI accession No.	Reference sequence accession No.	Identities between nucleotide (%)
Avo7	*L. theobromae*	LC790046	MN114216	98.73
Non pathogen				
Avo1	N/A	–	–	–
Avo2	N/A	–	–	–
Avo2.1	*N. sphaerica*	LC790042	MN053315	98.58
Avo2.3	*Neopestalotiopsis* sp.	LC790040	MN813055	93.75
Avo3.1	*Pestalotiopsis* sp.	LC790041	MK512478	96.35
Avo4	*P. ardesiacum*	LC790044	OQ571335.1	98.24
Avo5	N/A	–	–	–
Avo6	*Neopestalotiopsis* sp.	LC790045	OR609370	99.32
Avo8	N/A	–	–	–
Avo12	N/A	–	–	–
Avo13	N/A	–	–	–
Avo_14	*N. parvum*	LC790047	MK781982	97.32
Avo15	N/A	–	–	–
Avo_16	*P. ardesiacum*	LC790048	MK495813	96.97
Avo_17	N/A	–	–	–

This study also found species from the Sporocadaceae group. Based on the macroscopic observations, the colony of two isolates (Avo2.3 and Avo6) had white and cottony mycelium with a change in colour to slightly yellow as the culture age increased. Their conidia had three/four septa with conidiophores that were versicolour, ellipsoid, fusoid, and slightly curved at the edges (Figure 5). The apical and basal conidia are hyaline, and the middle cell of the conidia is dark brown.

Molecular identification and phylogenetic analysis. The PCR analysis amplified the ITS, TEF1 and LSU region generated fragments. A total number of 17 recovered isolates were included for the phylogenetic reconstruction using LSU-rDNA sequences. The 17 obtained sequences were deposited in the gene bank (Table 1).

The BLAST comparison results of the two pathogen isolates, Avo3.2 and Avo7 isolates, with the reference isolates in the ITS, LSU and TEF1 regions showed that the Avo3.2 isolates had 100% genetic similarity with *L. theobromae* in all three regions, while isolate Avo7 has 99.85% homology with *L. krabiensis,* and 98.73% with *L. theobromae* in the LSU and TEF1 region, respectively. Isolate Avo7 could not be identified in the ITS region in this study (Table 1).

After performing a BLAST comparison and phylogenetic analysis of the isolates in this study alongside those in GenBank (Table 1, Figure 6), all the isolates except Avo3.2 and Av07 were grouped into three common classes of endophytic fungi, namely Sordariomycetes, Eurotiomycetes and Dothideomycetes. The isolates belonging to the Sordariomycetes class were Avo2.3 and Avo6 (*Neopestalotiopsis* sp.), Avo1, Avo2, Avo8 and Avo15 (*Colletotrichum* sp.), Avo2.1 (*Nigrospora* sp.), Avo3.1 (*Pestalotiopsis* sp.), Avo12 (*Nemania* sp.). The isolates grouped in Dothideomycetes include Avo5 (*Lasiodiplodia* sp.), Avo14 (*Neofusicoccum* sp.), Avo4 and Avo16 (*Pseudofusicoccum* sp.) and Avo17 (*Neoroussoella* sp.), Isolate A13 from the BLAST results is *Penicillium* sp., the Eurotiomycete fungus group.

The pathogenicity test showed that the Avo7 and Avo3.2 isolates can induce necrosis on avocado stem branches. Based on the phylogenetic analysis, the 28S rDNA sequence alone is not sufficient to precisely identify the Lasiodiplodia species, therefore, a phylogenetic tree analysis for *Lasiodiplodia* spp. was measured based on the analysis of the combination of the 28S rDNA and TEF1 sequences using the Mega 11 software and the results showed that the isolates Avo7 and

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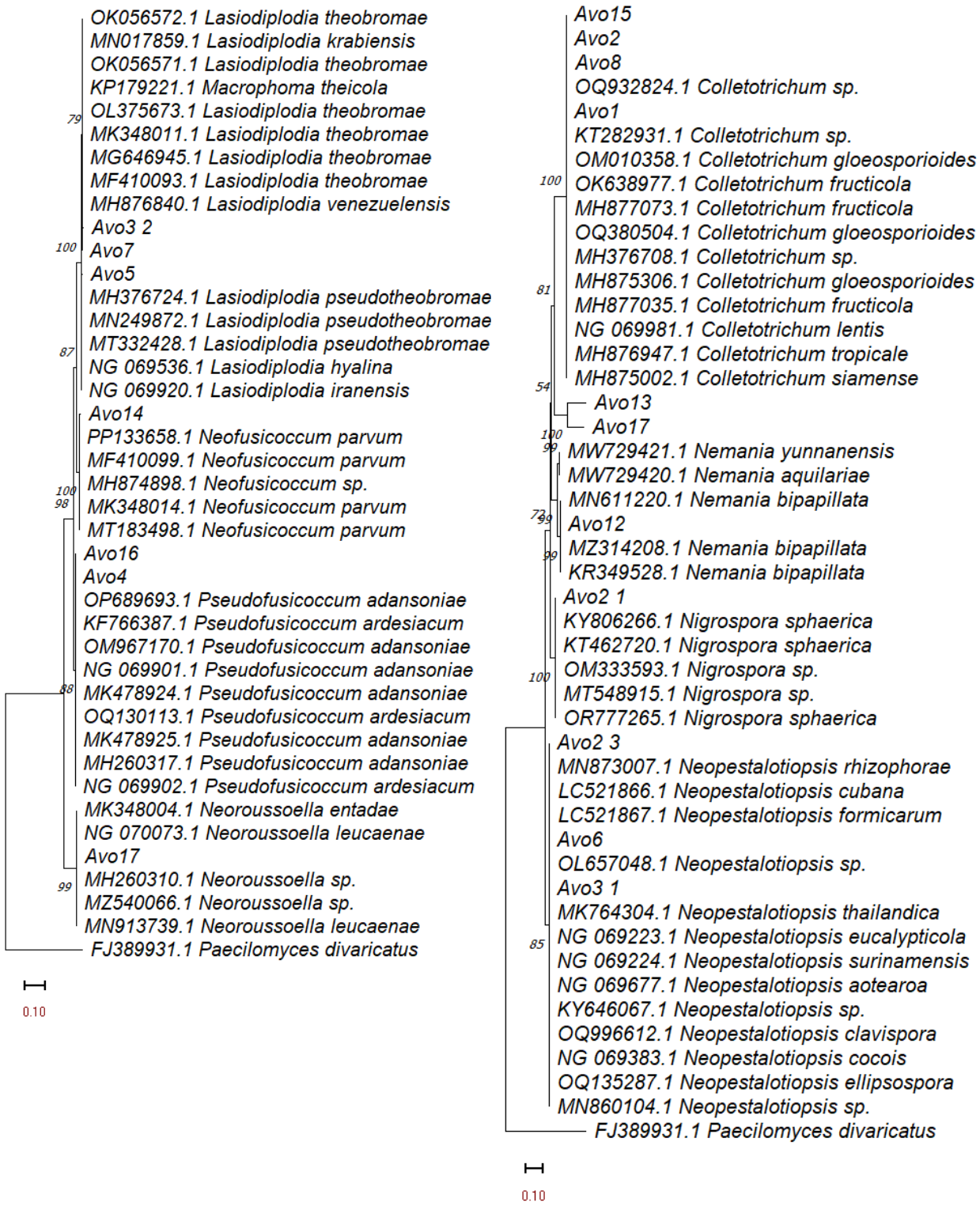

Figure 6. The neighbour joining tree for all the isolates isolated from the dieback disease symptoms of avocados based on the LSU rDNA sequences

the isolates were grouped into Dothideomycetes (A), Sordariomycetes (B) and Eurotiomycetes (C); the bootstrap values in the phylogenetic tree analysis were above 50 for each branch. The percentage of isolate clustered was with 1 000 bootstrap replications; the isolate *Paecilomyces divaricatus* (FJ389931) was an outgroup

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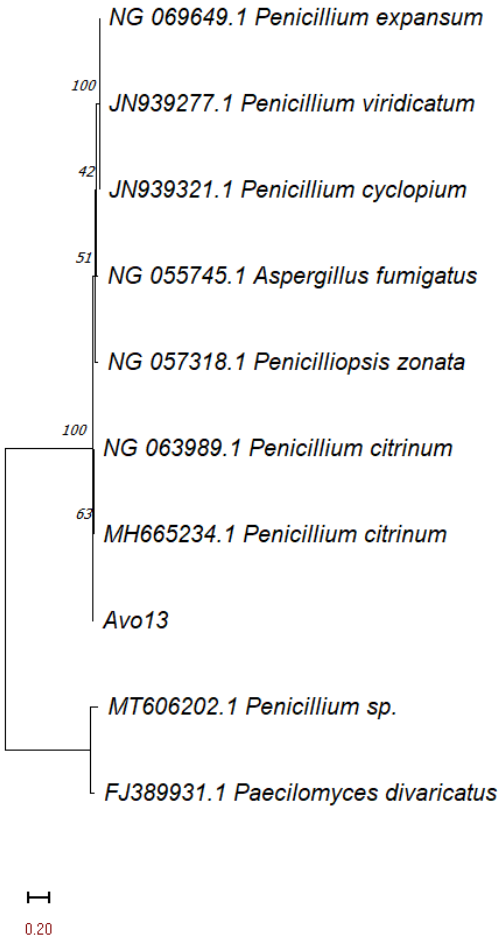

Figure 6. To be continued...

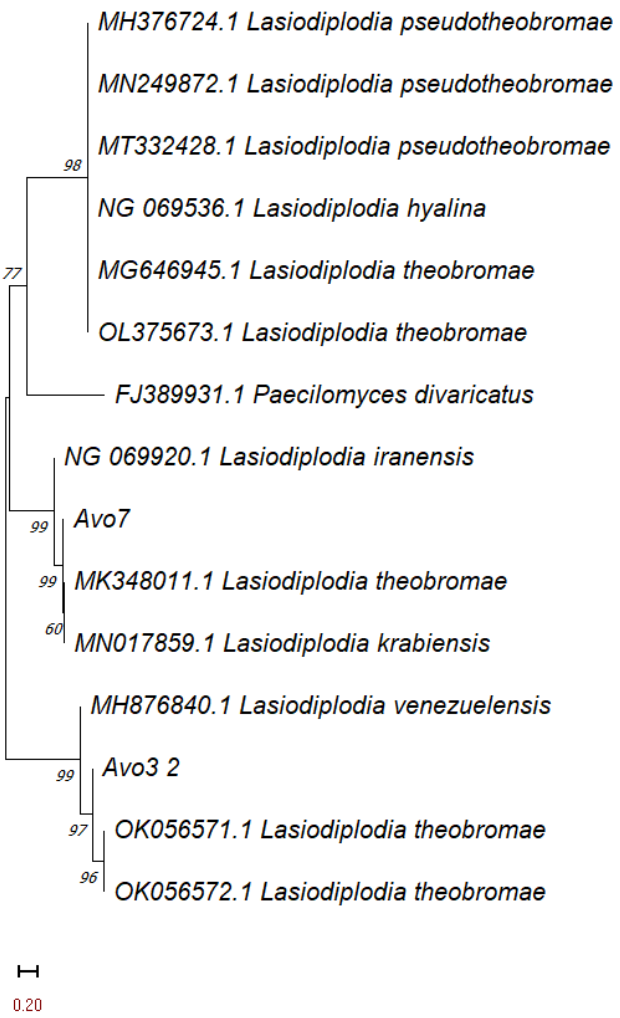

Figure 7. The phylogenetic tree of the Avo7 isolate and representative *Lasiodiplodia* spp. constructed with neighbour joining method used the combined TEF1 and LSU datasets

The phylogenetic tree was generated in the program Mega 11. The percentage of isolates clustered was measured by 1 000 bootstrap replications. The isolate *Paecilomyces divaricatus* (FJ389931) was added as an outgroup

Avo3.2 are closely related to the *L. theobromae* group (Figure 7).

DISCUSSION

The decline of avocado production is a major consideration worldwide. In Indonesia, it was reported that the major pathogen reducing the avocado production is Phytophthora disease and most of the studies are focused on how to control the Phytophthora disease and yet there are no reports of dieback disease. Typical symptoms of dieback disease were particularly found throughout the avocado plantations at the STIFAI Sumani experimental orchards in Solok district, West Sumatra.

Therefore, identification of the disease is particularly crucial, where other producing countries have been focused on investigating this disease. The study's aim was to isolate and characterise the pathogen and fungi that are associated on avocado plants showing dieback disease symptoms. The characterisation of the dieback disease occurs from the necrotic bark, red-brown wood, and discoloration of xylem (Auger et al. 2013). Based on the pathogenicity test results, the Avo7 and Avo3.2 isolate induce a necrosis symptom on avocado seedlings one month after inoculation (Figure 5). The phylogenetic analysis confirmed that this species is *L. theobromae* which has recently been reported as the causal pathogen of dieback and canker disease on avocados

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in China, Spain, and Colombia (Arjona-Girona et al. 2019; Ramírez-Gil & Peterson 2019; Qiu et al. 2020) while *Lasiodiplodia* spp. were reported as the pathogen of avocado plants in Mexico, Chile, and Peru (Valencia et al. 2018; Valle-De la Paz et al. 2019; Rodríguez-Gálvez et al. 2021). *Lasiodiplodia* species, representing the genus of the Botryosphaeriaceae family, exhibit as latent endophytes, pathogens or saprotrophs (de Silva et al. 2019). In this study, *L. theobromae* can cause necrosis symptoms after one month, but further observations are needed to prove that this species can cause progressive disease symptoms that are detrimental to plants.

This study revealed that there are several species of fungi isolated from the avocado branches. Mosquera et al. (2023) stated that the fungal groups Sordariomycetes, Eurotiomycetes, and Dothideomycetes are associated with avocados. Furthermore, it was explained that the abundance of all these groups, except for Eurotiomycetes, increased in the plants affected by root rot disease (Solis-Garcia et al. 2021) and lenticel-like damage (Mosquera et al. 2023). These fungi are thought to have antagonistic potential.

Based on the morphological and molecular analyses, the species associated in the stem branches of avocado plants belong to Sordariomycetes which the Glomerellaceae group is one of them. The Glomerellaceae family predominantly colonises in the avocado stem and root (Challacombe et al. 2019). *Colletotrichum* spp., a species grouped in Glomerellaceae, is a worldwide distributed and wide host range fungal species which has been isolated from various plant species as a fungal pathogen and endophyte (Jayawardena et al. 2016). In this study, the isolates were isolated from branches of avocado plants showing dieback diseases. The pathogenicity test results show that *Colletotrichum* spp., e.g., *C. tropicale, C. fruticola* and *Colletotrichum* spp. have no pathogenicity on the inoculated-avocado seedlings. Several studies reported that *C. fruticola* and *C. tropicale* are endophytic species (Talhinhas & Baroncell 2023; Norphanphoun & Hyde 2023), while *C. gloeosporoides, C. tropicale* were found in association with anthracnose diseases (Peng et al. 2013).

The fungal species, such as *N. parvum* (Botryosphaeriaceae family) and *N. sphaerica*, were identified in this study, which have been reported to be associated with avocado branches and the xylem (Shetty et al. 2016; Pérez-Martinez et al. 2018). Two other species of Sordariomycetes isolated from the avocado stem are *Neopestalotiopsis* sp. and *Pestalotiopsis* sp. Two other species in the *Neopestalotiopsis* genus, *N. siciliana* and *N. rosae* are recognised as pathogens causing stem lesion and dieback disease in avocados. However, in the present study, the pathogenicity of *Neopestalotiopsis* sp. and *Pestalotiopsis* sp. was not observed on the avocado seedlings and necrosis at the inoculation point did not extend more than 2 mm and did not cause other symptoms. Furthermore, *N. bipapillata, N. leucaenae* and *P. ardesiacum* were successfully isolated from the avocado branches in this study.

CONCLUSION

In this study, the most commonly found fungal isolates associated with dieback symptoms in avocado trees at the STIFAI plantation, West Sumatra, Indonesia, belong to the groups Botryosphaeriaceae, *Penicillium* spp., *Pestalotiopsis* spp., and *Colletotrichum* spp. Two tested isolates (Avo7 and Avo3.2) were able to cause necrosis symptoms in the avocado seedlings. These two isolates are fast-growing fungi, with whitish colonies resembling cotton that turn greenish-grey. The molecular identification of the ITS and LSU regions and phylogenetic analysis revealed that both isolates are of the species *L. theobromae.*

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