



REVIEW ARTICLE

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Characterization of the Pathogenic Fungus *Fusarium oxysporum* Causing Root Rot in Avocado Plants (*Persea americana* Mill.) in Buleleng Regency

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Abstract

Avocado (*Persea americana* Mill.) is one of the horticultural commodities with significant economic value, and its production has increased in Buleleng Regency. Root rot disease is of major constraint in avocado production, alongside weather and climate factors. This study aims to determine the species of pathogenic fungi causing root rot that infect avocado plants in Buleleng Regency, based on morphological characteristics, molecular analysis, and phylogenetics. The methods used in this study include: (1) observation and sampling of plants; (2) isolation of pathogenic fungi; (3) pathogenicity tests; (4) macroscopic and microscopic morphological identification; (5) molecular identification through PCR using ITS1-ITS4 primers; and (6) phylogenetic analysis using MEGA 11 software version 11.0.13. We successfully obtained isolates from symptomatic avocado root samples collected from five locations, namely Wanagiri, Gesing, and Madenan. The results of the pathogenicity tests showed that the Gesing isolate was able to produce symptoms similar to root rot in the field on healthy avocado seedlings. Based on morphological characteristics, as well as molecular and phylogenetic analyses, we identified the isolate as *Fusarium oxysporum*. This study is the first scientific report on the characterization of pathogenic fungi associated with root rot disease in avocado plants in Buleleng Regency. The information obtained can serve as a reference for diagnosing and monitoring of root rot disease in avocado plants, as well as a basis for developing appropriate disease management strategies.

Keywords: ITS, Morphology, Pathogenicity, Phylogeny, Sequencing

1. Introduction

Indonesia, as a tropical country, has great potential for developing various types of horticultural commodities, especially fruits. One type of fruit with high economic value that is currently popular among the public is avocado (*Persea americana* Mill.). The variety of avocado cultivars and increasing market demand make avocado cultivation a promising and profitable business opportunity.

National avocado production in Indonesia has increased from 2019 to 2023, with figures as follows: 461,613 tons (2019), 609,049 tons (2020), 669,260 tons (2021), 865,780 tons (2022), and 874,046 tons (2023) (BPS, 2024). Avocado plants have been widely distributed across almost all provinces in Indonesia, including Bali. Several areas in Bali are known as avocado production centers, such as Gianyar, Bangli, Badung, and Buleleng.

Currently, avocado cultivation in Buleleng Regency has expanded across various sub-districts, with most farmers using polyculture systems. However, rising avocado production in Buleleng faces threats from root rot, which can disrupt plant growth and reduce productivity. Various soil-borne pathogens, including *Phytophthora* sp., *Fusarium* sp., and *Pythium* sp can cause root rot in avocado plants. Infection of the root system can interfere with water and nutrient absorption, leading to decreased plant vigor, chlorosis, and wilting. As the disease progresses, symptoms may include leaf drop, dieback at the tips of the plant, and inhibited fruit formation, ultimately reducing yield and fruit quality. In severe cases, soil-borne pathogens can kill plants and threaten the sustainability of avocado production (Ramírez-Gil et al., 2017; Kavroulakis et al., 2024).

One of the pathogens that has been widely recognized

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as a cause of root rot is *Phytophthora cinnamomi* Rands (Álvarez et al., 2023). However, avocado root rot can also involve other pathogens such as *P. tropicalis*, *P. kelmanii*, *Phytophthora vexans*, and *P. chamaecephalon*, which have also been reported to be associated with the disease. (Madhu et al., 2025). In addition to the oomycetes group, several species of the genus *Fusarium* have also been reported to be associated with root rot disease in avocado plants. Olalde-Lira et al. (2020) reported *Fusarium oxysporum* and *F. solani* as pathogens causing avocado root rot in Michoacan, México, based on morphological and molecular characterization and pathogenicity tests. Another study in Nayarit, México, also showed that avocado root rot can involve several pathogen groups, including the *Fusarium oxysporum* species complex, *F. solani* species complex, *Phytophthora cinnamomi*, and other soil-borne pathogens. (Rosales et al., 2026). The diversity of pathogens reported in various regions indicates the importance of identifying the cause of root rot in a particular avocado planting location.

Identification of pathogenic fungi that cause disease can be performed using morphological and molecular approaches. Morphological identification is a fundamental method in fungal recognition, based on observing colony characteristics and microscopic structures. This provides an initial indication of fungal identity before further molecular analysis (Maharani et al., 2025). The results of these observations can then be supported by molecular identification based on DNA sequence analysis. The ribosomal DNA region, particularly the Internal Transcribed Spacer (ITS), is widely used for species identification and determining phylogenetic relationships (Takamatsu, 1998; Larena et al., 1998).

Both morphological and molecular approaches have been employed in studies of avocado root rot in various regions, such as those conducted by Olalde-Lira et al. (2020) and Rosales et al. (2026). However, based on a literature review, scientific information regarding the causal fungi of root rot in avocado plants in Buleleng Regency is not yet available.

This study was conducted to identify the fungal species causing root rot in avocado plants in Buleleng Regency through morphological and molecular characterization, as well as phylogenetic analysis.

2. Material and Methods

The research was conducted from July to October 2025. Plant sampling was carried out in five villages in Buleleng Regency, Bali Province, namely Wanagiri Village (8.212119° S, 115.123264° E; ±1.037 masl), Gesing (8.291703° S, 115.070183° E; ±1.056 masl), Munduk (8.266761° S, 115.065447° E; ±780 masl), Madenan (8.152772° S, 115.301050° E; ±616 masl), and Umajero (8.306222° S, 115.057592° E; ±983 masl). Isolation of pathogenic fungi causing root rot and *in vitro* antagonist

tests were conducted at the Plant Disease Laboratory, while greenhouse experiments were conducted at the Greenhouse of the Buleleng Regency Agriculture Service. Molecular identification of pathogenic fungi was carried out through analysis of the ribosomal DNA (rDNA) region using *Internal Transcribed Spacer* (ITS) markers with the forward primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and the reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3').

The tools used in this study include Petri dishes, Erlenmeyer flasks, glass beakers, autoclaves, laminar airflow, centrifuges, vortex mixers, thermal cyclers, micropipettes, Eppendorf tubes, microscopes, microscope slides, coverslips, UV transilluminators, gel electrophoresis systems, stationery, and millimeter blocks.

The materials used include avocado plant samples infected with pathogenic fungi, PDA media, PDB media, healthy avocado plant seeds, alcohol, sodium hypochlorite, spirits, liquid nitrogen, Cethyl Trimethyl Ammonium Bromide (CTAB) buffer, agarose gel powder, TE buffer, universal primer ITS1 (F), Primer ITS4 (R), 70% ethanol, PCR Master Mix, β-mercaptoethanol, chloroform isoamylalcohol, sodium acetate (CH₃COONa), nuclease-free water, DNA markers, DNA dyes, as well as four isolates of antagonistic fungi *Trichoderma* sp. and two isolates of *Gliocladium* sp. from the collection of the Plant Disease Laboratory, Faculty of Agriculture, Udayana University.

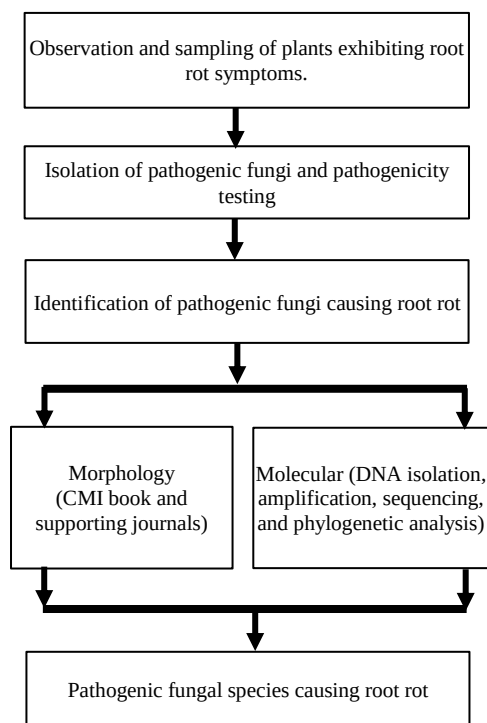


Figure 1 Research Flow diagram

Observation and sampling of symptomatic plants
Root sampling was conducted using *purposive sampling* on avocado plants showing root rot symptoms

such as wilting, yellowing leaves, leaf fall, and rotting roots. Samples were taken from five avocado plantations in Buleleng Regency: Wanagiri, Gesing, Munduk, Umajero, and Madenan villages.

Isolation of pathogenic fungi and pathogenicity testing

The obtained root samples were cut into 1×1 cm pieces, washed and sterilized with 70% alcohol and rinsed with sterile distilled water, then grown on PDA medium and incubated at 25- 30 °C for 3 - 5 days. The mycelium growing from the edge of the tissue was transferred to new PDA medium to obtain pure cultures, which were then used in the pathogenicity test.

2.1. Pathogenicity Test

Pathogenicity tests were conducted to ensure that fungal isolates obtained from avocado plant roots with root rot symptoms were capable of causing the same disease symptoms in healthy plants. Pure isolates of the pathogenic fungus were first grown in *Potato Dextrose Broth* (PDB) medium, then inoculated onto healthy avocado plant seedlings by injuring the base of the stem and roots of the plant by piercing the base of the stem with a sterile needle and cutting four plant roots. Next, 20 mL of the pathogenic fungal culture in PDB medium was poured around the roots. We compared the symptoms that appeared with those of root rot in avocado plants in the field. Isolates that were able to cause similar symptoms were then re-isolated on PDA media and continued with morphological and molecular identification.

2.2. Morphological identification

We observed pure cultures of pathogens from pathogenicity tests macroscopically and microscopically. Morphology observed included colony shape, colony color, hyphal structure, conidial shape, and conidial size.

2.3. Molecular identification

Molecular identification was performed of analyzing the Internal Transcribed Spacer (ITS) region using universal primers, namely ITS1 as the forward primer (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 as the reverse primer (5'- TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The stages of molecular identification include DNA isolation, DNA amplification, sequencing, and phylogenetic analysis. We sent the amplified products to First Base (Malaysia) for sequencing. The sequencing results were analyzed using the *Basic Local Alignment Search Tool* (BLAST) feature available on the *National Center for Biotechnology Information website* (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the level of similarity of the isolate sequence with the reference sequence in GenBank. The phylogenetic tree was constructed using MEGA 11 Version 11.0.13 with the Neighbor Joining method based on the Tamura 3-parameter nucleotide substitution model, with a bootstrap test of 1000

replications, to support the determination of pathogen species identification.

3. Results and Discussion

Based on field observations at five avocado plantations in Buleleng Regency, avocado plants infected with root rot generally exhibit initial symptoms of yellowing, wilting, drying, and leaf fall. Symptoms in the crown are characterized by drying of the tips of the branches, which progresses downward, eventually leading to branch death. In advanced cases, dismantling the root area reveals rot that begins in the small roots or root hairs and then spreads to the main root.

Isolation of pathogenic fungi and pathogenicity testing

Isolation of symptomatic avocado root samples revealed three fungal isolates with distinct colony growth characteristics. We named these isolates based on their location of origin: the Wanagiri isolate, the Madenan isolate, and the Gesing isolate. We then tested these isolates for pathogenicity.

The results of the pathogenicity test showed that the Gesing isolate caused root rot symptoms similar to those observed in avocado plants in the field.



Figure 2 Symptoms of root rot resulting from inoculation in the pathogenicity test. (a) Avocado seedlings treated with sterile water (control), (b) symptoms that appear on avocado seedlings inoculated with Gesing isolate

Observations showed that avocado seedlings inoculated with the Gesing isolate exhibited symptoms in the crown of the plant, including yellowing, dullness, and wilting of the leaves (Figure 2). As the infection progressed, seedling growth was stunted, and the leaves began to dry and fall off. The symptoms observed in this pathogenicity test are consistent with a report by Ramírez Gil et al. (2014), which states that pathogen infection in young plants can cause stunted growth, decreased plant vigor, yellowing of the leaves, and wilting. As the infection progresses, wilting symptoms can worsen, followed by leaf fall and ultimately plant death.

Avocado seedlings showing signs of infection, when removed from their growing medium, showed signs of

damage to their root systems (Figure 3). Fibrous roots were few, blackish-brown in color, soft, and rotting. In contrast, seedlings inoculated with the Wanagiri, Madenan, and control isolates showed no disease symptoms in either the crown or the root system.

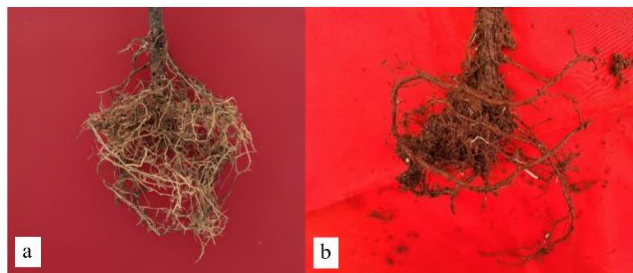


Figure 3 Roots of avocado seedlings removed in the pathogenicity test. (a) Roots of avocado seedlings in control treatment (roots look healthy), (b) roots of avocado seedlings inoculated with Gesing isolate (roots look rotten and have few fibrous roots)

3.1. Morphological Identification

The Gesing isolates from the pathogenicity test were re-isolated on PDA medium to obtain pure cultures, then identified based on macroscopic and microscopic characteristics.

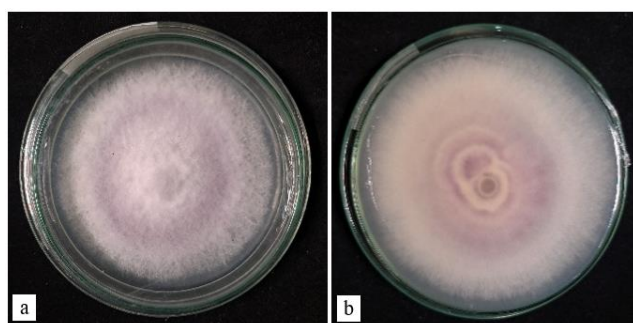


Figure 4 Colony of pathogenic fungus isolates Gesing isolated from avocado plants with root rot symptoms in the pathogenicity test. (a) top view of the colony; (b) bottom view of the colony.

The results of observations based on macroscopic characters show that the Gesing isolate has a purplish-white colony, a smooth cotton-like surface, and the edges of the colony appear flat and spread radially (Figure 4). Based on microscopic observations with a 400x magnification microscope, it can be seen that the Gesing isolate has septate and hyaline hyphae (Figure 5). Microconidia are oval in shape with 0-1 septum, measuring $2.8\text{--}3.5 \times 9.5\text{--}11.5 \mu\text{m}$, and are grouped. Macroconidia are curved like a crescent with 3-5 septums, measuring $3\text{--}5.5 \times 25\text{--}50 \mu\text{m}$. Chlamydospores are formed at the tip of the hyphae, are round (*globose*) with relatively thick cell walls, and are formed in pairs.

Based on the macroscopic and microscopic morphological characteristics obtained, the isolate is

suspected to belong to the *Fusarium* sp. fungal group, characterized by the presence of vegetative spores, namely microconidia, macroconidia, and chlamydospores. The morphological characters in this study are in line with the report of Walida et al. (2019), stating that *Fusarium* sp. has a white colony shape and a cotton-like mycelium shape. However, some colonies have white colonies with purple or orange centers. The study of Wanjiku et al. (2020) on *Fusarium* associated with *stem-end rot* in avocado plants in Kenya showed that *Fusarium* has microconidia of $11.2\text{--}19.9 \mu\text{m} \times 4.5\text{--}8.4 \mu\text{m}$ without partitions and macroconidia measuring $22.1\text{--}43.9 \mu\text{m} \times 5.1\text{--}12.5 \mu\text{m}$ with 2-3 partitions.

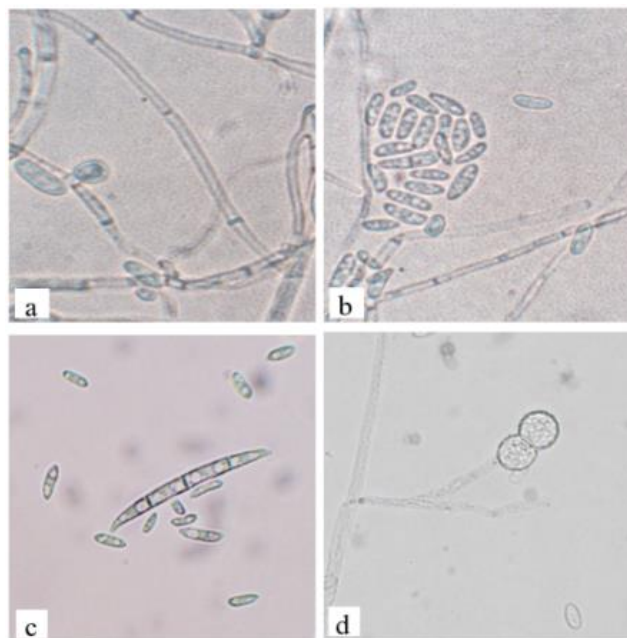


Figure 5 Microscopic identification results of Gesing isolates. (a) Septate hyphae, (b) Microconidia, (c) Macroconidia, and (d) Chlamydospores

3.2. Molecular Identification

Molecular identification begins with DNA amplification of the Gesing isolate using PCR with the universal primer pair ITS1-ITS4. In fungi, rDNA sequences are found in the cell nucleus and mitochondria. The rDNA regions are separated by a boundary called a spacer. In fungal rDNA, conservative regions include the 18S, 5.8S, and 28S rRNA coding genes, with an Internal Transcribed Spacer (ITS) region between them (Gomes et al., 2002; O'Brien et al., 2005).

The ITS region is frequently used in molecular phylogenetic studies because it is highly copy-numbered in the nuclear genome. It also has a high PCR amplification success rate and is easily sequenced in both *forward* and *reverse directions*. Furthermore, the ITS region shows a high degree of variation, allowing it to differentiate closely related fungal species and support the identification of new species (Hariri et al., 2021; Letchuman, 2018).

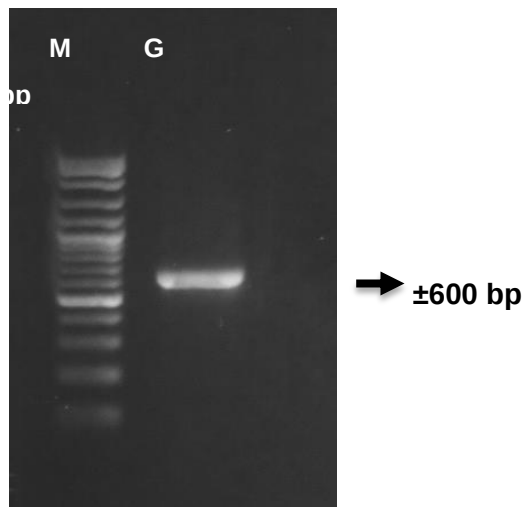


Figure 6 Visualization of ITS amplification results of Gesing isolate

The electrophoresis visualization showed that the Gesing isolate DNA was successfully amplified with a fragment size of ± 600 bp (Figure 6). This fragment size is in accordance with the results of research by Nirmaladevi et al. (2016), who reported that amplification of the ITS region of *Fusarium oxysporum* using ITS1-ITS4 produced fragments of around 600bp, and Singha et al. (2016) obtained ITS fragments ranging from 380-620bp in several *Fusarium* isolates, including *F. oxysporum*. These studies showed that the ITS1-ITS4 primer pair amplified the ITS region, including ITS1, the 5.8S rDNA gene, and ITS2 in

Fusarium, and produced PCR products that could be sequenced to obtain DNA sequences.

We analyzed the sequencing results of the Gesing isolate by comparing the nucleotide sequence with reference sequences available in the GenBank (NCBI) database using the BLAST feature. of selected the reference sequence with the highest Query Cover and Percent Identity values, which indicate the level of sequence coverage and nucleotide similarity between the Gesing isolate and the reference isolate.

Based on the search results in the GenBank (NCBI) database, the Gesing isolate sequence showed a high level of similarity with several reference sequences. *F. oxysporum*. The results of the identity matrix analysis showed that the Gesing isolate had nucleotide sequence similarity ranging from 90.9% to 99.7% to the selected reference sequences (Table 1). This value is in line with the research of Wanjiku et al. (2020), which reported ITS sequence similarity of 99% in *F. oxysporum* isolates. This high sequence similarity indicates that the Gesing isolate is genetically closely related to the *F. oxysporum* reference sequence. Based on the ITS markers used, the Gesing isolate isolated from avocado roots with root rot symptoms in Buleleng Regency was identified as *F. oxysporum*. Further molecular analysis was carried out of constructing a phylogenetic tree to determine the kinship relationship between the Gesing isolate sequence and the reference sequence available in the GenBank database.

Table 1 Results of identity matrix analysis of nucleotide sequences of Gesing isolates with reference sequences in GenBank

GenBank reference isolates	Accession Number	Country of origin	(%) Identity of Gesing Isolate
<i>Fusarium oxysporum</i>	OK560835	Malaysia	99.7
<i>Fusarium oxysporum</i>	PP434803	Indonesia	99.7
<i>Fusarium oxysporum</i>	PZ516038	India	99.7
<i>Fusarium oxysporum</i>	PQ481988	China	91.1
<i>Fusarium oxysporum</i>	OP456681	Türkiye	90.9
<i>Fusarium oxysporum</i>	HG529205	Italy	90.9
<i>Fusarium oxysporum</i>	KT876656	Great Britain	90.7
<i>Fusarium oxysporum</i>	KY114145	Argentina	91.1
<i>Fusarium oxysporum</i>	MK114163	Ecuador	91.1
<i>Fusarium oxysporum</i>	OR913029	Spanish	91.1
<i>Ilyonectria liriodendra</i>	PV440765	Italy	85.8

F. oxysporum reference sequences, namely OK560835 (Malaysia), PP434803 (Indonesia), and PZ516038 (India), with 99% bootstrap support (Figure 7). Similar results were reported by Bektas & Kusek (2019), who obtained 99% bootstrap support in the grouping of *F. oxysporum* isolates, while Liu et al. (2019) obtained 60% bootstrap support in the *F. oxysporum* clade based on phylogenetic analysis of the ITS region. The separation of several *F. oxysporum* sequences into different clades in this study indicates the existence of ITS sequence variations between isolates. Meanwhile, *Ilyonectria liriodendra*, PV440765, separated

from all *F. oxysporum* isolates and occupied the outgroup position, indicating the most distant kinship relationship with the Gesing isolate. A similar grouping pattern is in accordance with the results of the study by Chakrapani et al. (2023), which showed that *F. oxysporum* isolates based on ITS sequence analysis were divided into two groups, with one isolate forming a separate group from the other isolates. These results indicate that variations in the ITS region can produce different grouping patterns among *F. oxysporum* isolates.

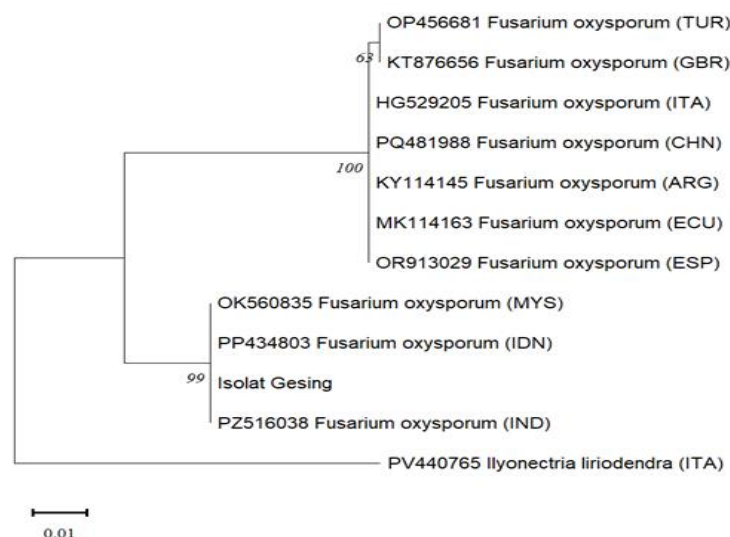


Figure 7 Phylogenetic tree of Gesing isolates based on ITS-rDNA sequences using the *Neighbor-Joining method* in MEGA 11 software version 11.0.13

4. Conclusion

This study successfully identified *Fusarium oxysporum* as the pathogenic fungus causing root rot in avocado plants in Buleleng Regency, based on pathogenicity tests, morphological characteristics, and

molecular and phylogenetic analyses. This study is the first scientific report of its kind in the region and can serve as a foundation for diagnosing and developing management strategies for avocado root rot.

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