J. Trop. Plant Pests Dis.
Vol. 25, No. 1, March 2025
Pages: 32–43

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.12532-43

SHORT COMMUNICATION

Detection and identification of fungi causing strawberry wilt disease in North Sumatra, Indonesia

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Manuscript received: 16 November 2023. Revision accepted: 10 January 2024. Available online: 6 February 2025.

ABSTRACT

Strawberry (*Fragaria* sp.) is primarily grown in temperate and some subtropical countries. With the expansion of fruit commodities in Indonesia, including the introduction of foreign cultivar, strawberry has been increasingly cultivated locally. In North Sumatra, strawberry cultivation, mainly for agritourism, is concentrated in Karo Regency, Berastagi District. This study aimed to detect and identify fungi responsible for wilt disease in strawberry plants across several areas of Berastagi, North Sumatra. This research was conducted from July 2022 to May 2023 at the Plant Disease Laboratory, Faculty of Agriculture, Universitas Sumatera Utara. The study followed Koch's postulates: the pathogen was isolated and purified from symptomatic plants, then inoculated into healthy plants. Infected plants exhibiting the same symptoms as the initial sample were subsequently re-isolated, purified, and identified at the molecular level. The results confirmed that the causal agent of wilt disease in Daulu (Rini Colia Strawberry, Esy Azera Strawberry) and Dolat Raya (Sonakmalela Strawberry, Alea Strawberry, Sembiring Gurky Strawberry) was *Fusarium oxysporum.*

Key words: Fungal detection, *Fusarium*, strawberry, strawberry wilt

INTRODUCTION

Strawberry (*Fragaria* sp.) is one of the horticultural crops that grow in tropical countries. Its geographical distribution is extensive, spanning various continents, including America, Europe, Asia, and Indonesia (Oktarina et al., 2017) Strawberry cultivation has been established in several regions of Indonesia, such as North Sumatra, West Sumatra, West Java, Malang, Bali, and Sulawesi.

Indonesia's strawberry production fluctuated between 2017 and 2021, with production figures as follows: 12,224 tons; 8,528 tons; 7,499 tons; 8,350 tons; and 9,859 tons, respectively. The corresponding harvested areas were 582 ha, 618 ha, 543 ha, and 682 ha for both 2020 and 2021 (Irjayanti et al., 2022). The decline in strawberry production in Indonesia is influenced by various factors, including disease infection such as wilt disease.

Strawberry wilt disease is a soil-borne disease that can cause a significant decrease in production, with losses of up 95% (Sari et al., 2018). The main symptoms of this disease include a change in leaf color from green to yellowish-green and reddish-brown, starting at the edges of the leaves (Koike & Gordon, 2015). Additionally, symptoms at the base of the stem include browning of the stem, blackish-brown roots, and stunting, which typically occur during the day (Sari et al., 2018).

Understanding the rhizosphere environment is critical because it plays a key role in the interaction between plants and soil-borne pathogens, influencing both the development of diseases like strawberry wilt and strategies for their management. Rhizosphere microorganisms contribute to various processes, such as nutrient cycling, soil formation, plant growth promotion, microbial activity regulation, and biological control (Prayudyaningsih et al., 2015). However, some microbes in the strawberry rhizosphere can also act as pathogens, leading to production losses and even plant death. Pathogenic species include *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Colletotrichum* sp., and *Verticillium* sp. (Putra et al., 2020).

Based on initial survey of this research at several locations in Daulu and Daulat Raya villages, plant showing symptoms of strawberry wilt disease have been detected. To date, no research has been conducted on the detection and identification of fungi causing wilt disease isolated from the rhizosphere of strawberry plants in the Berastagi Areas.

Consequently, research is needed to detect

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and identify pathogenic fungi in the rhizosphere of strawberry plants. This study aimed to identify the causal pathogens of the wilt disease affecting strawberry plants in several areas of Berastagi, North Sumatra. Since the identification of plant pathogens is the first step in plant disease control, accurate detection and identification can enhance disease management strategies (Afandi et al., 2021). The findings of this study could assist farmers in selecting the most effective management strategies to control the fungal pathogens responsible for the disease.

MATERIALS AND METHODS

Research Site. The research was conducted at the Plant Disease Laboratory, Faculty of Agriculture, Universitas Sumatra Utara, Medan.

Research Implementation. This research applied the concept of Koch's postulates, which state that 1) The pathogen must be present in plants exhibiting disease symptoms; 2) The pathogen can be isolated from diseased plants and grown in pure culture; 3) The pathogen, when inoculated into healthy plants, must cause symptoms similar to the original disease; 4) The pathogen must be re-isolated from the inoculated plants (Koch, 1982).

Sampling of Symptomatic Plants with Wilt Disease. Sampling of symptomatic plants was conducted in Daulu and Dolat Raya villages across five garden locations (Table 1). Three plants showing wilt symptoms were randomly selected from each location. The five chosen locations were agritourism areas in Berastagi. Symptoms observed in the plant samples included browning at the base of the stem, yellowish-green or reddish-brown discoloration of the leaves starting from the edges, stunting of the plants, and decay occurring during the day (Henry et al., 2017).

Isolation of Stems and Roots from Plants with Wilt Symptoms. Stems and roots were carefully washed under running tap water and then cut into 0.5–1.0 cm pieces. These pieces were disinfected using 70% alcohol for approximately 15–30 s, rinsed with sterile distilled water, and dried on paper towels in a laminar flow cabinet. The surface-sterilized plant parts were then cultured on PDA (Potato Dextrose Agar) media. The samples were placed at three points in a petri dish and incubated until fungal mycelium grew on the culture media. The resulting fungal growth was subsequently transferred to new culture media to obtain pure cultures (Juber et al., 2014).

Koch's Postulate Test. Pathogenic fungal strains isolated from wilted strawberry plants were tested on healthy, one-month-old strawberry plants. Inoculation was performed using the dilution series method, where the pure culture was dissolved in 10 mL of distilled water and diluted to create a suspension of 10^6 CFU/mL. A 10 mL aliquot of the suspension was poured around the roots of each healthy plant. Healthy plants that developed symptoms after inoculation, identical to those observed in the field, were re-isolated, and the fungi were purified. Pure cultures with the same characteristics as the initial pathogenic fungi were confirmed as the causal agents of wilt disease in strawberry plants (Sari et al., 2018).

Macroscopic, Microscopic, and Identification Observations of Wilt Disease-Causing Fungi. Macroscopic observation of pathogenic fungi causing wilt disease was conducted by directly examining the development of fungal colonies on petri dishes. Observations included the shape, color, texture, and elevation of each coloy.

Microscopic observations were performed by aseptically taking a pure fungal culture using a preparation needle and placing it on a glass slide. A drop of Methyl Blue dye was added to enhance visualization the microscopic structures of the fungi, which were identified using the method described by Leslie & Summerell (2008). Observation were made under a compound microscope (Binocular microscope Carl Zeiss Primo Star) at 40× magnification.

For photographing fungal structures, the

Table 1. Sampling locations of wilt-symptomatic strawberry plants in Berastagi, North Sumatra

Village	Garden	Land area	Coordinate point
Daulu	Rini Colia Strawberry	1 Ha (polyculture)	3°12'22" N, 98°32'01" E
	Esy Azera Strawberry	1 Ha (polyculture)	3°12'23" N, 98°32'00" E
Dolat raya	Sonakmalela Strawberry	1 Ha (polyculture)	3°11'54" N, 98°32'24" E
	Alea Strawberry	0.5 Ha (monoculture)	3°10'44" N, 98°33'02" E
	Sembiring Gurky Strawberry	1 Ha (polyculture)	3°11'58" N, 98°32'20" E

microscope was carefully adjusted, including proper lighting and centering the condenser below the stage. A series of exposures was taken, and the focus was incrementally adjusted between shots to capture clear images.

Molecular Identification. Molecular identification was performed at the Genetic Science Indonesia Tangerang Laboratory using DNA barcoding. Fungal DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) following the manufacturer's protocol. The concentration and quality of the extracted DNA were determined using NanoDrop Spectrophotometry (Thermo Scientific) (Table 2), and the DNA was stored at -20 ºC until further use.

DNA amplification was carried out using the 2× MyTaq HS Red Mix kit (Bioline, BIO-25048) with a PCR Master Mix (Table 3). The ITS region within the 16S–23S rRNA was amplified using the ITS1 Primer (TCCGTAGGTGAACCTGCGG) and ITS4 Primer (TCCTCCGCTTATTGATATGC) (White et al., 1990). The PCR conditions included an initial denaturation at 95 ºC for 1 min (1 cycle), followed by 35 cycles of denaturation at 95 ºC for 10 s, annealing at 52 ºC for 15 s, and extension at 72 ºC for 15 s. PCR products were stored at 4 ºC.

The amplified ITS gene products were electrophoresed on a 1% agarose gel in TBE buffer at 50 volts for 30 min. The DNA bands were visualized under a UV transilluminator, and DNA fragments were compared with a 100 bp DNA ladder (Figure 1). Two-way sequencing was performed using the Sanger sequencing method via capillary electrophoresis. BLAST analysis was conducted on GenBank (NCBI) to identify the fungal isolates.

The ITS gene sequences of the five fungal isolates analyzed in this study were reviewed and checked using ChromasPro 1.5, then assembled into contigs using the CAP3 Sequence Assembly Program

Table 2. DNA quantification results using Nanodrop

No.	Isolates	Concentration	$A_{260/280}$	$A_{260/230}$	Volume (µL)
1	Rini Colia Strawberry	30.50	1.70	0.85	50
2	Esy Azera Strawberry	21.80	1.70	0.55	50
3	Sonakmalela Strawberry	25.70	1.66	0.87	50
4	Alea Strawberry	15.60	1.70	0.59	50
5	Sembiring Gurky Strawberry	29.30	1.66	0.30	50

Table 3. PCR Master Mix

Component	1 × 25 (µL)	Final concentration
Dd H2O	until 25 µL	1×
My Taq HS Red Mix (2×)	12.5	1×
10 µM ITS1 Primer (5'–TCCGTAGGTGAACCTGCGG–3')	1	0.4 µM
10 µM ITS4 Primer (5'–TCCTCCGCTTATTGATATGC–3')	1	0.4 µM
DNA Template	X	1.5–75 ng

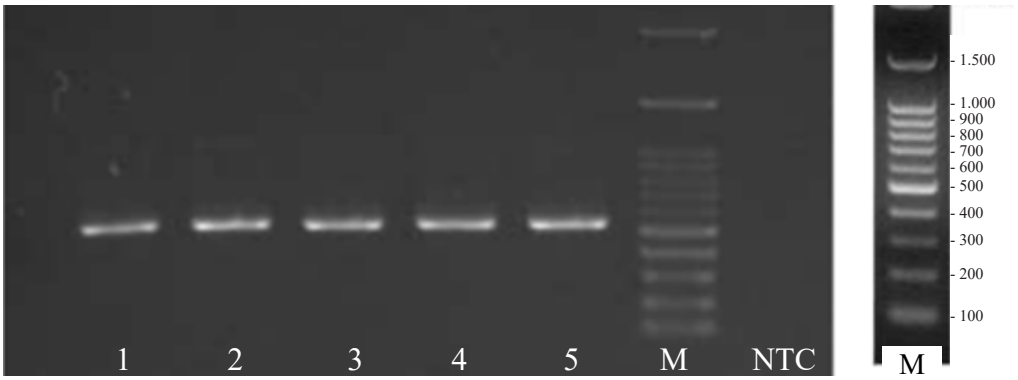


Figure 1. Electrophoresis results of the amplification products. 1= Isolate from Rini Colia Strawberry (RC-S); 2= Isolate from Esy Azera Strawberry (EA-S); 3= Isolate from Sembiring Gurky Strawberry (SG-S); 4= Isolate from Sonakmalela Strawberry (S-S); 5= Isolate from Alea Strawberry(A-S); M= Marker.

(Huang & Madan, 1999).

Sequence Alignment and Phylogenetic Analysis. All sequences generated from this study, along with those obtained from the GenBank database (nine isolates of *Fusarium oxysporum* as ingroups and *Colletotrichum acutatum* as an outgroup), were aligned using the default settings of CLUSTAL W, version 2.0 (Larkin et al., 2007; Thompson et al., 1994). Complete alignments are provided in Appendices D.1, D.2, and D.3.

Phylogenetic analysis of the sequences was conducted using Molecular Evolutionary Genetics Analysis (MEGA) software, version 11.0 (Tamura et al., 2021). The neighbor-joining method was employed to assess evolutionary relationships within the data (Saitou & Nei, 1983). Bootstrap analysis was performed with 1000 replicates to ensure the reliability of the phylogenetic tree.

Observation Parameters. The observation parameters of this study included the following: a). The disease symptoms observed in the field must match the symptoms induced after the Koch's postulate test; b). The colony morphological characteristics (macroscopic observation) must remain consistent before and after the Koch's postulate test; c). The fungal morphological characteristics (microscopic observation) must remain consistent before and after the Koch's postulate test.

RESULTS AND DISCUSSION

Symptoms of Strawberry Wilt Disease. Symptoms of wilt disease were characterized by leaf discoloration, turning yellowish-green and reddish-brown, starting from the edges. The base of the stem became brown, the plant became stunted, and wilting occured during the day (Table 4). Wilting is the primary symptom of *Fusarium* wilt disease because fungal penetration into the plant roots leads to the disruption of water-conducting xylem vessel (Yadeta & Thomma, 2013).

Based on the field observations, most plants exhibited chlorosis, characterized by leaf discoloration to yellowish-green and reddish-brown. Henry et al. (2017) differentiated two syndromes of *Fusarium* wilt in strawberries: chlorosis (*yellows-fragariae*) and wilting (*wilt-fragariae*). Consequently, the five *Fusarium* isolates from this study are categorized as *wilt-fragariae*. These two syndromes, 'yellow' and 'wilt', are caused by independently evolved pathogen genotypes that do not share a pathogenicity chromosome (Henry et al., 2017).

A study by Gargita et al. (2020) showed that strawberry wilt is characterized by red spots on the leaves, which spread rapidly, causing the leaves to dry out and appear burnt. The symptoms also include withering of older leaves and brown to reddish-brown discoloration of the internal vascular and cortical tissues of plant crowns, ultimately reducing fruit production (Koike & Gordon, 2015). The fungal pathogens responsible for strawberry wilt enter the plant through the roots, colonize the root cortex, and then invade the xylem (Triasih et al., 2023).

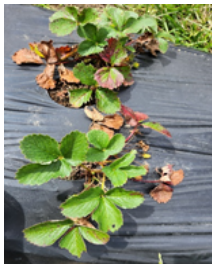
Macroscopic Observation of Isolates Collected from Wilt-Symptomatic Plants in the Field. Based on the isolation results from five sample locations, 15 fungal isolates collected from wilt-symptomatic strawberry plants in the field. Out of these 15 isolates, five had similar macroscopic and microscopic characteristics and were suspected of causing wilt disease (Table 5). The five isolates that met the criteria for similar macroscopic and microscopic characteristics were subjected to Koch's postulate test, while the remaining ten isolates were excluded because they did not fulfill the requirements of Koch's postulate.

Macroscopic and microscopic observations revealed that these fungi exhibited macroscopic characteristics such as a circular colony form, a white surface color with a purple base, and a smooth, cotton-like colony texture (Cottony) with raised colony elevations. Microscopic observations showed that these fungi had non-septate hyphae and two forms of conidia. The macroconidia consisted of three or more septa, were curved, and had pointed ends, while the microconidia had 1–2 septa and were ovoid or oval in shape (Table 5). The morphology, growth, and development of the macroconidia and microconidia were consistent with descriptions of *Fusarium oxysporum* Schlechtend emend. Snyder & Hansen (1940).

Gargita et al. (2020) states that *Fusarium* spp. grown on PDA media exhibit white mycelium that can turn yellow or cream-colored. Some *Fusarium* spp. isolates can produce pinkish, slightly purple, red, or deep purple pigments under certain conditions. The study by Triasih et al. (2023) showed that microconidia of *Fusarium* spp. infecting strawberries had 1–2 septa abd were ovoid, whereas macroconidia had 3–6 septa and were oval with pointed tips or spindle-shaped.

The fungi causing strawberries wilt disease in the Berastagi region, based on macroscopic and microscopic observations, closely resembled *Fusarium oxysporum,* which is known to cause wilt in strawberries. However, to confirm these findings,

Table 4. Symptoms of strawberry wilt disease in the field

No	Location	Reference	Symptoms on the field
1	Rini Colia Strawberry	 Koike and Gordon (2015); Sari et al. (2018)	
2	Esy Azera Strawberry	 Koike and Gordon (2015); Sari et al. (2018)	
3	Sonakmalela Strawberry	 Koike and Gordon (2015); Sari et al. (2018)	
4	Alea Strawberry	 Koike and Gordon (2015); Sari et al. (2018)	
5	Sembiring Gurky Strawberry	 Koike and Gordon (2015); Sari et al. (2018)	

Koch's postulat test was conducted.

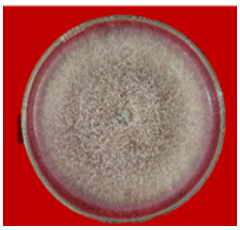
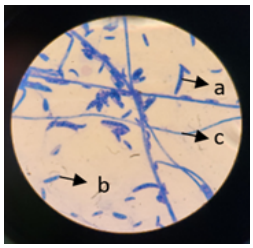
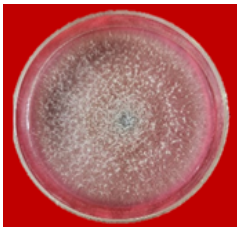
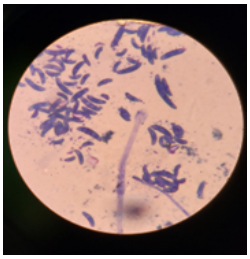
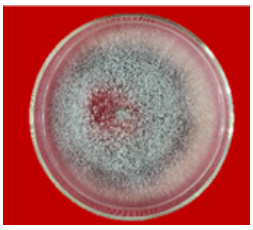
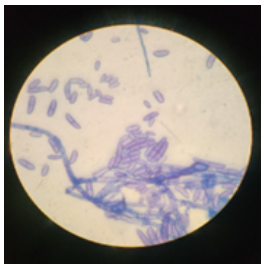
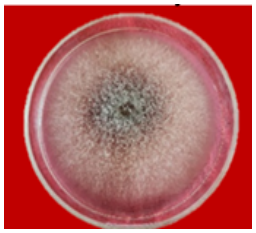
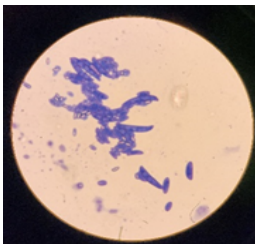
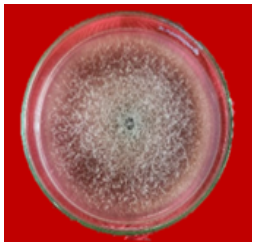
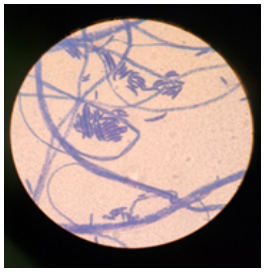
Koch's Postulate Test. Based on the results of Koch's postulate test, the initial symptoms appeared in the fourth week after inoculation, manifesting as a change in leaf color to reddish-yellow, starting from the edges (Table 4). The symptoms observed in inoculated plants were identical to those seen in field-infected strawberry plants, and these symptoms were absent in healthy strawberry plants.

The wilt symptoms and fungal characterization in this study align with the findings of Triasih et al. (2023), who reported the characterization of *Fusarium* wilt in strawberries from different regions in Indonesia.

The disease caused by *Fusarium* spp. is characterized by stunting, reduced productivity, necrosis, and drying of leaves and petioles, as well as crown discoloration. In severe cases, plants eventually collapse and die (Koike & Gordon, 2015).

Fusarium wilt disease rapidly infects strawberry plants, often causing sudden wilting and death. Like other vascular wilt pathogens, *Fusarium* infects

Table 5. Macroscopic and microscopic observations

No	Macroscopy					Microscopy
	Figure	Colony form	Colony color	Colony texture	Colony elevation	Figure
1	 Rini Colia Strawberry	Circular	White surface; purple base	Smooth like cotton (*Cottony*)	Raised	
2	 Esy Azera Strawberry	Circular	White surface; purple base	Smooth like cotton (*Cottony*)	Raised	
3	 Sonakmalela Strawberry	Circular	White surface; purple base	Smooth like cotton (*Cottony*)	Raised	
4	 Alea Strawberry	Circular	White surface; purple base	Smooth like cotton (*Cottony*)	Raised	
5	 Sembiring Gurky Strawberry	Circular	White surface; purple base	Smooth like cotton (*Cottony*)	Raised	

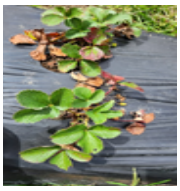
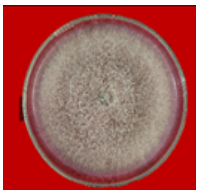
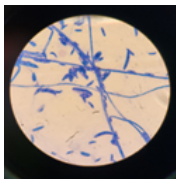
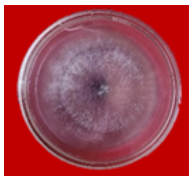
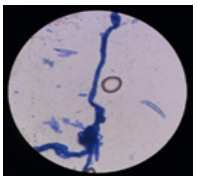
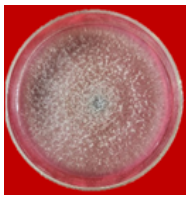
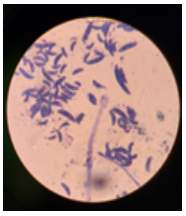
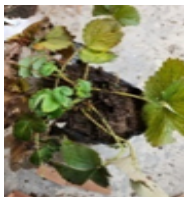
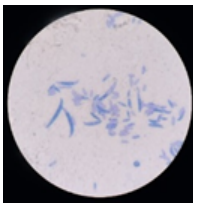
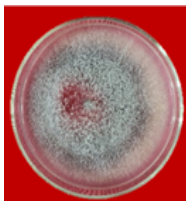
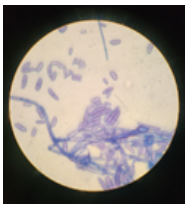
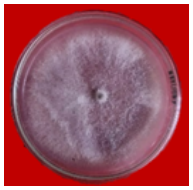
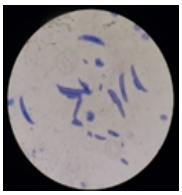
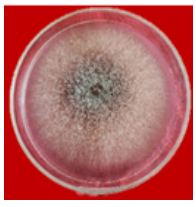
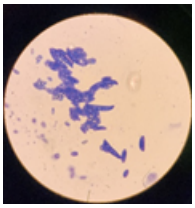
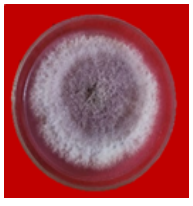
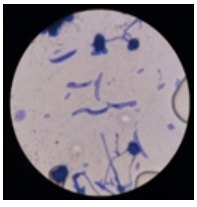
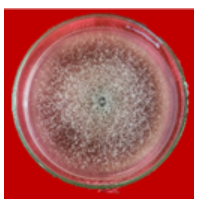
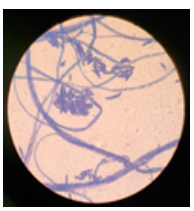
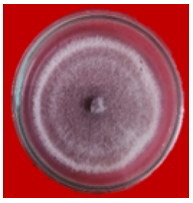
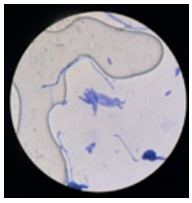
Description: a. Macroconidia; b. Microconidia; c. Hyphae

healthy plants by penetrating root tips, root wounds, or lateral roots before spreading intracellularly into the xylem. It produces microconidia that colonize and block vascular vessels, disrupting water and nutrient translocation. Consequently, this leads to epinasty, yellowing of the lower leaves, progressive wilting, and ultimately, plant death (Boba et al., 2020).

Observation of Disease Symptoms, Macroscopic, and Microscopic Characteristics, and Identification of Plant Samples Tested by Koch's Postulates. This study is the first report on the detection and identification of the cause of wilt disease in strawberry farms in North Sumatra, specifically in five farms in the Berastagi area. Based on the results obtained, macroscopic observations showed similarities before and after Koch's postulate test. These observations, summarized in Table 6, revealed consistent colony characteristics, including a circular shape, a white surface, a purple center at the base, and a cotton-like texture.

Microscopic observations also indicated similar

Table 6. Observations of disease symptoms, macroscopic & microscopic before and after Koch's Postulate test

No	Before Koch's Postulate Test			After Koch's Postulate Test		
	Disease Symptoms	Macroscopic Structure	Microscopic Structure	Disease Symptoms	Macroscopic Structure	Microscopic Structure
1						
2						
3						
4						
5						

fungal structures before and after conducting Koch's postulate test. The microconidia were ovoid or oval, containing 1–2 septa, while the macroconidia were twisted or crescent-shaped with pointed tips and had three or more septa. Based on these results, the isolated fungi were identified as the same fungi responsible for strawberry wilt disease in the field. This finding confirms the principles of Koch's postulates.

Macroscopic and microscopic observations indicated that these fungi exhibited characteristics consistent with the genus *Fusarium*. The fungal colonies had a white surface with a purple base, and the macroconidia were twisted or crescent-shaped with pointed ends, consisting of three or more septa. The microconidia were ovoid or oval-shaped, containing 0–2 septa (Figure 2A). This is in accordance with Barnett & Hunter (1998), who stated that *Fusarium* spp. produces cotton-like mycelium, which can appear pink, purple, or yellow in culture media. *Fusarium* spp. also has multi-celled macroconidia that are slightly curved with pointed tips, and single-celled microconidia that are ovoid or oval-shaped (Figure 2B).

Fusarium wilt disease on strawberries in Bali exhibits microscopic features where the microconidia are ovoid, and the macroconidia are spindle-shaped, oval with sharp tips, and contain 2–6 septa (Figure 2C) (Gargita et al., 2020).

Molecular Identification. Based on the molecular identification results of the five isolates and sequence data (Table 7), which were compared with those in GenBank using the BLAST program, the isolates from Daulu village (Rini Colia Strawberry (RS-C) and Esy Azera Strawberry (EA-S)) and Dolat Raya village (Sonakmalela Strawberry (S-S), Alea Strawberry (A-S), and Sembiring Gurky Strawberry (SG-S)) were identified as *Fusarium oxysporum,* with 100% sequence similarity.

The dendogram, constructed from the 16S-23S rRNA gene ITS region sequences of the five fungal isolates from this study, was built by comparing the sequences to nine isolates of *F. oxysporum* from the GeneBank database. *Colletotrichum acutatum*, which is not a member of the *F. oxysporum* species, was used as an outgroup. The sequences lenght is approximately 524 nucleotides, obtained from the 16S-23S rRNA ITS region. The phylogenetic tree, calculated based on the Neighbor-Joining (NJ) algorithm, revealed a single group corresponding to the phylotype scheme (Figure 3). All fungal isolates from this study formed a single cluster with the *F. oxysporum* isolates from the GenBank database, due to the 100% sequence similarity. It has been advised that ≥97–100% sequence similarity (i.e., up to 3% sequence divergence) should be used for assigning a species name from BLAST search identifications of fungi using ITS sequences against GenBank (Raja et al., 2017).

This research observed the Fusarium wilt symptoms in five garden locations in Daulu and Dolat Raya villages. Most infected plants exhibited chlorosis, characterized by leaf discoloration, turning yellowish-green to reddish-brown. After confirming the five fungal isolates through Koch's postulate test, their macroscopic and microscopic characteristics, as well as the symptoms observed in tested plants, were consistent with those of the initial fungal isolates obtained from the fields. Further molecular identification confirmed that all isolates belonged to *Fusarium oxysporum*.

CONCLUSION

Based on macroscopic and microscopic observations, molecular identification, and Koch's postulate test, *Fusarium oxysporum* has been confirmed as the causal pathogen of strawberry wilt

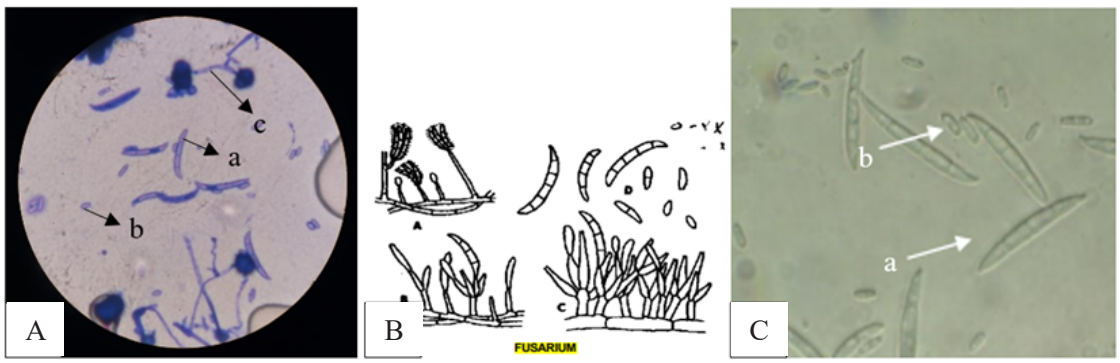
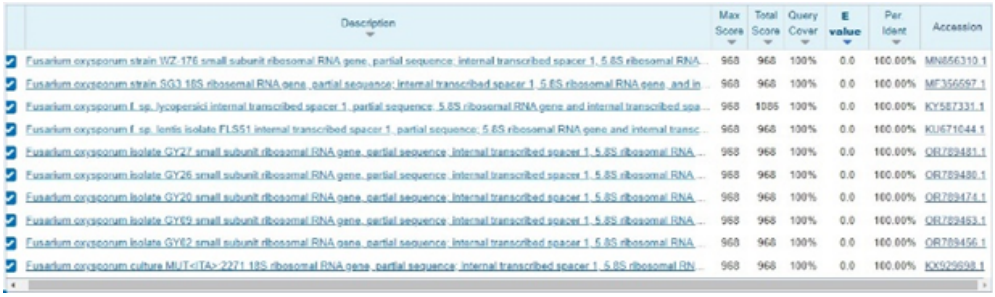
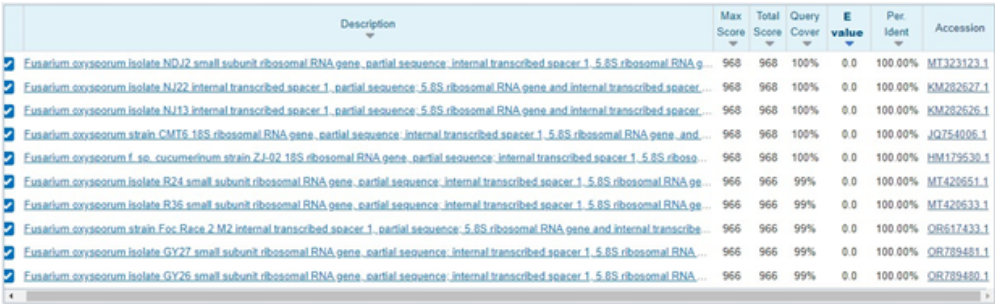
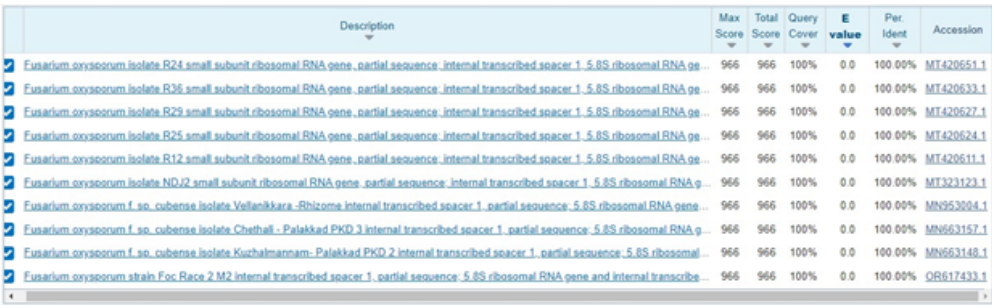
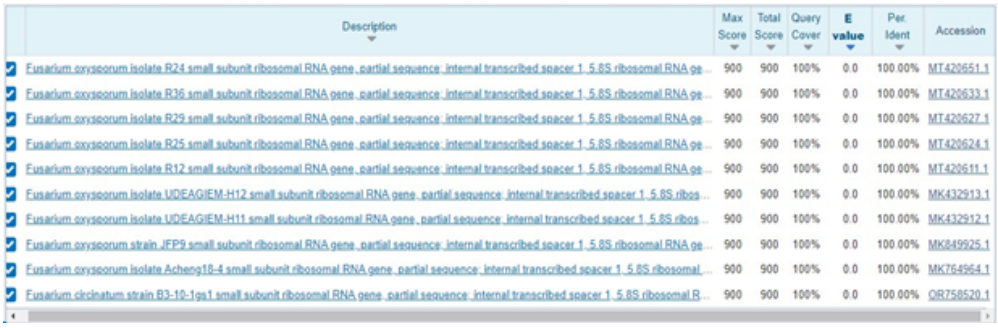


Figure 2. Microscopic structure of Fusarium. A. Microscopic structure of the fungus causing strawberry wilt disease. B. Illustration of Fusarium by Barnett & Hunter (1998); C. Microscopic structure of *Fusarium* spp. by Gargita et al. (2020). a. Macroconidia; b. Microconidia; c. Hyphae.

Table 7. Molecular Detection Results Based on NCBI Blast Search

No	Isolate	Results
1	Rini Colia Strawberry (RC-S)	
2	Esy Azera Strawberry (EA-S)	
3	Sonakmalela Strawberry (S-S)	
4	Alea Strawberry (A-S)	
5	Sembiring Gurky Strawberry (SG-S)	

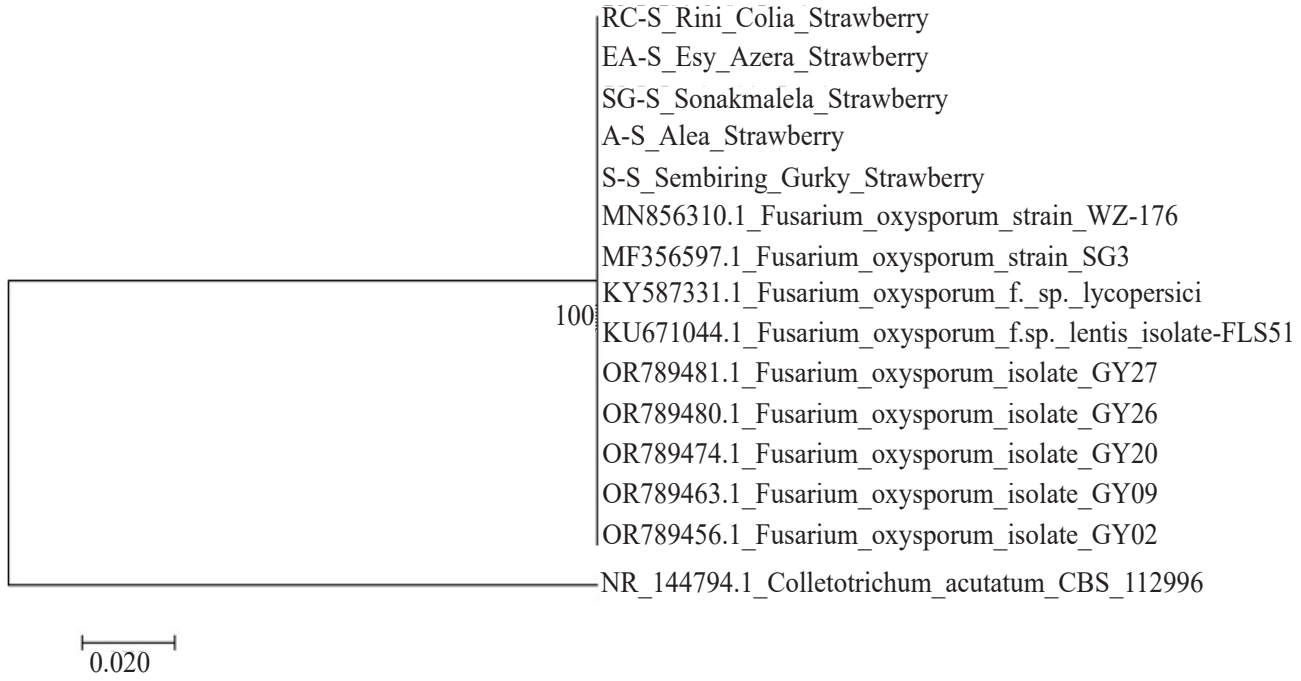


Figure 3. Neighbour-joining tree showing phylogenetic relationships among sample strains compared with *Fusarium oxysporum* isolates based on 16S-23S rRNA ITS region sequences. *Colletotrichum acutatum* was used as the outgroup. Scale bar represents one nucleotide sustitution per 100 nucleotides. Bootstrap values of 1000 resampling are shown at branch points.

disease in the Berastagi Region, specifically in Daulu village (Rini Colia Strawberry, Esy Azera Strawberry) and Dolat Raya village (Sonakmalela Strawberry, Alea Strawberry, and Sembiring Gurky Strawberry). To our knowledge, this is the first report of *Fusarium* wilt disease caused by *F. oxysporum* on strawberry plants in North Sumatra.

ACKNOWLEDGMENTS

No acknowledgments are applicable for this study.

FUNDING

This study received no external funding.

AUTHORS' CONTRIBUTIONS

CPS and IS conceptualized and designed the experiment. CPS conducted sampling and observation of strawberry wilt symptoms in various areas of Berastagi, North Sumatra. CPS and IS provided feedback on the research process and contributed to manuscript preparation. All authors have read and approved the final version of the manuscript.

COMPETING INTEREST

The authors declare that they have no competing interests related to the publication of this manuscript.

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