

FIRST REPORT OF *Fusarium sulawesiense* CAUSING FUSARIUM WILT OF MELON MANIS TERENGGANU (*Cucumis melo* VAR. *Inodorus* CV. MANIS TERENGGANU 1) IN MALAYSIA

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Abstract: Fusarium wilt is a devastating disease affecting cucurbit crops worldwide and its emergence in new regions can have significant agricultural implications. This study presents the first report of Fusarium wilt caused by *Fusarium sulawesiense* in the Melon Manis Terengganu cultivar. The fungi were identified through characteristic symptoms, pathogen isolation, and molecular confirmation. Pathogenicity tests established the virulence of *F. sulawesiense*. The result showed that among 18 fungi isolated at 10 parts of the plant for three seasons, fungi from the wilted plant with ID MMTF1 were primarily found in all parts of the plant every season. Fungus ID MMTF1 was confirmed by molecular identification as *F. sulawesiense*. Pathogenicity by seed germination indicated that severity was 100% from day one to day seven. Plant infection tests by spraying and wound inoculation showed that the *F. sulawesiense* spraying method caused the most rapid effect on the leaf, which became wilted and the leaf stem split compared to wound inoculation. The disease incidence (DI) study indicated that wilt and diseased vascular (stem rot) caused about 61.67% (DI) and 35.83% of plant deaths in monsoon season. This study emphasises the need for ongoing monitoring and research to safeguard the melon industry in Malaysia.

Keywords: *Fusarium sulawesiense*, Melon Manis Terengganu, Fusarium wilt.

Introduction

Melon Manis Terengganu (*Cucumis melo* var. *Inodorus* cv. Manis Terengganu 1) is a popular melon in Malaysia, which is exclusively cultivated in the Terengganu state. It was first introduced in 2015 and belongs to the Cucurbitaceae family (Azmi *et al.*, 2022; Jusoh *et al.*, 2022). It has a smooth yellow peel, orange-like salmon flesh colour, and a sweet taste with Brix between 12% to 15% (Amiza & Loo, 2020; Amiza *et al.*, 2022). Despite being valued for its sweet and refreshing flesh, Melon Manis Terengganu (MMT) production faces challenges from various pathogens, including *Fusarium* sp., known to cause wilt diseases in several plant species, especially melon. The condition is related to climate change, which is a favourable environment for infection.

Fusarium wilt is a devastating disease affecting many crops caused by ascomycete fungus, leading to significant yield losses and economic repercussions (Fernández-Cabanás *et al.*, 2022; Wang *et al.*, 2023). It is ranked fifth among the top 10 phytopathogens globally (Karthika *et al.*, 2022). The causal agents of Fusarium wilt are typically species within the genus *Fusarium* and they can be highly host-specific, posing a particular threat to crop diversity and food security. Zhao *et al.* (2021) have indicated that many species of *Fusarium* have been identified as plant pathogenic fungi, causing plant wilt, blight, rot, and canker diseases. It enters the plant via the roots and colonises the xylem vessels, hindering the plant from taking in regular amounts of water and eventually drying up. *Fusarium* sp. can persist

in soil for several years and spread through contaminated seed, soil, and plant debris (Mishra *et al.*, 2023). In recent years, novel *Fusarium* sp. have been identified, contributing to the complexity of these diseases.

This study presents the first report of *Fusarium sulawesiense* as the causative agent of Fusarium wilt in MMT. *F. sulawesiense* was first reported in Indonesia, causing Panama disease in small-holder banana plots (Maryani *et al.*, 2019). In 2022, *F. sulawesiense* was reported in China as the cause of soybean pot blight (Sun *et al.*, 2022). While in 2023, *F. sulawesiense* caused fusariosis in a pineapple in Thailand (Abeywickrama *et al.*, 2023). Understanding the presence of this pathogen in MMT crops is crucial for implementing effective disease management strategies and safeguarding the production of this highly valued fruit.

This report describes the symptoms, isolation, and identification of *F. sulawesiense*, along with preliminary insights into its pathogenicity and potential impact on melon cultivation in Malaysia. Furthermore, we discuss the significance of this discovery in the context of local agriculture and emphasise the importance of ongoing surveillance and research to protect the melon industry against emerging and re-emerging plant pathogens. This study contributes to the broader knowledge of Fusarium wilt diseases and underscores the need for continuous vigilance in managing emerging threats to crop health and sustainability in Malaysia.

Materials and Methods

Fungal Pathogen Collection

Samples were collected from the Melon Manis Terengganu field at Universiti Sultan Zainal Abidin, Besut Campus. The plants showed symptoms of Fusarium wilt from October 2020 to June 2021, which covers three seasons: Monsoon, transition, and drier. The plants ($n = 3$) were divided into seven sections, namely root, lower stem, middle stem, upper stem, leaf stem, leaf, and fruit stem. The fruit was divided

into three parts: Fruit skin, fruit juice, and fruit seed. Each section was cut into 2 cm x 3 cm pieces, washed with 1% sodium hypochlorite for 60 seconds, and rinsed with sterile distilled water three times for 60 seconds each (Wu *et al.*, 2023). The samples were then plated on Potato Dextrose Agar (PDA) (BD Difco) at room temperature. The fungi from each colony that grew on the media were subcultured multiple times until obtaining a single colony and stored at 4°C. The fungi were analysed by identifying them based on the part of the plant in which they were found. Additionally, they were compared across three different seasons. The most found fungi in each part of the plant that caused diseases were selected as the main pathogenic fungi.

Molecular Identification and Analyses

The selected fungi were identified through molecular characterisation using universal primers ITS1 and ITS4 to amplify the fungal ITS gene (Zhou *et al.*, 2022). The 25 mL reaction mixture consisted of purified gDNA obtained through an internally optimised protocol, 0.5 pmol of each primer, deoxynucleotide triphosphates (dNTPs) at a concentration of 200 µM each, 0.5 units of a heat-resistant DNA polymerase, PCR buffer provided by the manufacturer, and water. The PCR was performed with an initial denaturation cycle (98°C for 2 minutes), followed by 25 cycles (98°C for 15 seconds; 60°C for 30 seconds; 72°C for 30 seconds) for annealing and extension, and a final extension cycle (72°C for 10 minutes). The PCR products were purified by the standard PCR clean up method and the purified PCR products were subjected to bi-directional sequencing with universal primers M13F (-20) and M13R-pUC (-26) using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

To ensure the integrity of the sequences, amplicons were sequenced in both directions using the same primer pairs used for amplification. Consensus sequences were analysed and assembled using MEGA v. 11. The sequences were compared to the GenBank

Table 1: Accession numbers for *Fusarium* sp. from NCBI database

<i>Fusarium</i> sp.	Accession Numbers
<i>Fusarium sulawesiense</i>	OQ781864.1, KY436233.1
<i>Fusarium equiseti</i>	OQ732724.1, OQ658851.1
<i>Fusarium pernambucanum</i>	OQ708633.1
<i>Fusarium incarnatum</i>	OQ422647.1, OQ422646.1
<i>Fusarium oxysporum</i>	ON416887.1

database from NCBI (Table 1) using BLAST and search. Phylogenetic analyses were based on Maximum likelihood (ML) using MEGA v. 11 and the bootstrap support (BS) was determined automatically by the software using default parameters.

Morphology Identification

After determining the selected fungi at the molecular level, they were analysed based on their morphology. First, the chosen fungi were cultured on two types of agar: Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). The radial growth of the fungi was compared and the mycelium was observed using Scanning Electron Microscopy (SEM). The selected fungi were cultured on only one agar type (PDA or SDA) to observe their microscopic characteristics using a Leica microscope from Germany.

Pathogenicity and Plant Infection Test

Seed germination was used for pathogenicity by exposing the seed of MMT to the plate with pathogenic fungi compared with a control plate without pathogenic fungi; the data were recorded every day for seven days. Severity was calculated, where:

$$\% \text{ of Disease Suppression (DS)} = \frac{A - B}{A} \times 100 \quad (1)$$

A = Number of seeds germinate (NSG) in the control plate

B = NSG in pathogenic fungi plate

For the plant infection test, the fungus was prepared from the seven-day-old culture of test fungus on PDA and harvested with sterile

distilled water (10^6 CFU/ml) (Devi *et al.*, 2018). The leaves of MMT were sprayed-inoculated (conidial spray) and wound-inoculated (conidial droplet and mycelial plug). The severity of the disease was measured by scale of 0 = no symptoms; 1 = < 25% of leaves with symptoms; 2 = 26% to 50% of leaves with symptoms; 3 = 51% to 75% of leaves with symptoms; and 4 = 76% to 100% of leaves with symptoms (Karthika *et al.*, 2022).

Disease Incidence and Climate Change (Field Study)

The data on disease incidence was collected from November 2021 to January 2022 (Monsoon season) at the Melon Manis Terengganu field in the Universiti Sultan Zainal Abidin (UniSZA), Besut Campus. This period is known to be the most favourable season for pathogenic fungi and the data was collected from a population of 1,500 plants with a 95% confidence level and 5% margin error. A sample size of 360 plants was selected randomly and weather data was recorded from the Malaysian Meteorological Department (Karki *et al.*, 2022). The percentage of disease incidence was calculated by the following formula (Srivastava *et al.*, 2021):

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100 \quad (2)$$

Statistical Analysis

All the collected data was analysed using R-software. The fungal pathogen collection was conducted in triplicates, and the molecular identification analysis was carried out using the

software MEGA v. 11. Pathogenicity and plant infection tests were conducted in five replicates. The weather data was visualised using Tableau software.

Results and Discussion

Fungal Pathogen Isolation

Our study analysed the dynamics of *Fusarium* wilt in MMT by examining the present symptoms and fungal species. Figures 1 [a (i)] and [a (ii)] show wilted MMT plants while Figures 1 [a (iii)] and [a (iv)] illustrate symptoms similar to stem rot. A total of 18 single-colony fungi were found in 10 different parts of the plant after being separated from the mother plate. Figure 1 (b) shows the distribution of fungi in 10 parts of a plant throughout three seasons. Season 1 occurred from October 2020 to January 2021, the monsoon season. Season 2 was from February to April 2021, which was the transition period from the monsoon season to the drier season. Finally, season 3 took place from May to June 2021, which was the drier season. Across three seasons, the result indicated that MMTF1 found almost all parts of the plant, which were the root, lower stem, middle stem, upper stem, leaf stem, fruit stem, and fruit skin, followed by MMTF2. The co-occurrence of symptoms raises questions about the interplay between pathogens and their effects on MMT plants.

Table 2 presents results from the examination of 18 fungi across three seasons. Eight fungal species namely MMTF1, MMTF2, MMTF3, MMTF7, MMTF8, MMTF9, MMTF17, and MMTF18 consistently appeared, indicating their resilience and adaptability to change environmental conditions. MMTF1 was notably widespread across all plant sections and seasons, making it the most pathogenic fungi identified in the study and morphology found in Figures 1 [a (iii)] and [a (iv)]. Its unwavering presence and extensive distribution within MMT plants across different seasons suggest a significant and influential role in the manifestation and progression of *Fusarium* wilt in MMT plants.

Information about *F. sulawesiense* causing vascular wilt is alarming. Typically, *Fusarium oxysporum* is recognised as the causal agent of vascular wilt in various plant species (Hwang et al., 2022) and is highly toxic and pathogenic, reproducing asexually and surviving in soil for long periods. The mode of action of the pathogen involves the pathogen entering the root system and infecting the xylem vessels, which can lead to water and nutrient flow blockages. In addition, it causes the deposition of gums, thyllos, spores, and mycelia and visible discolouration of vascular tissue, leaf yellowing, wilt, and ultimately results in plant death (Srivastava et al., 2021; de Oliveira et al., 2023). It invades a plant's xylem, kills it, and releases further pathogens into the soil. The pathogen can exist as chlamydo-spores for an extended time, infecting nearby plants when conditions are favourable (Lin et al., 2023).

Molecular Identification and Analysis

We found that the main pathogenic fungus responsible was MMTF1, which was found in almost every part of the wilted plant. The morphology of this fungus was similar to that found in vascular disease, which set it apart from other fungi. MMTF1 has been identified as *Fusarium sulawesiense* based on the evidence presented in Figure 2. The phylogenetic tree shown in Figure 2 (b) illustrates the evolutionary relationships among the fungal isolates examined in this study. The tree features diverse clades and branches that cover a wide range of fungal taxa, providing insight into their unique species and interrelationships.

According to Sun et al. (2022), *F. sulawesiense* is classified within the *Fusarium incarnatum-equiseti* species complex (FIESC), which has over 30 pathogenic species, as reported by Wang et al. (2019). In a study by Xia et al. (2019), *F. sulawesiense* was identified as a phyllo-species FIESC 16 and formed a well-supported clade within the Incarnatum clade. FIESC has been detected in various

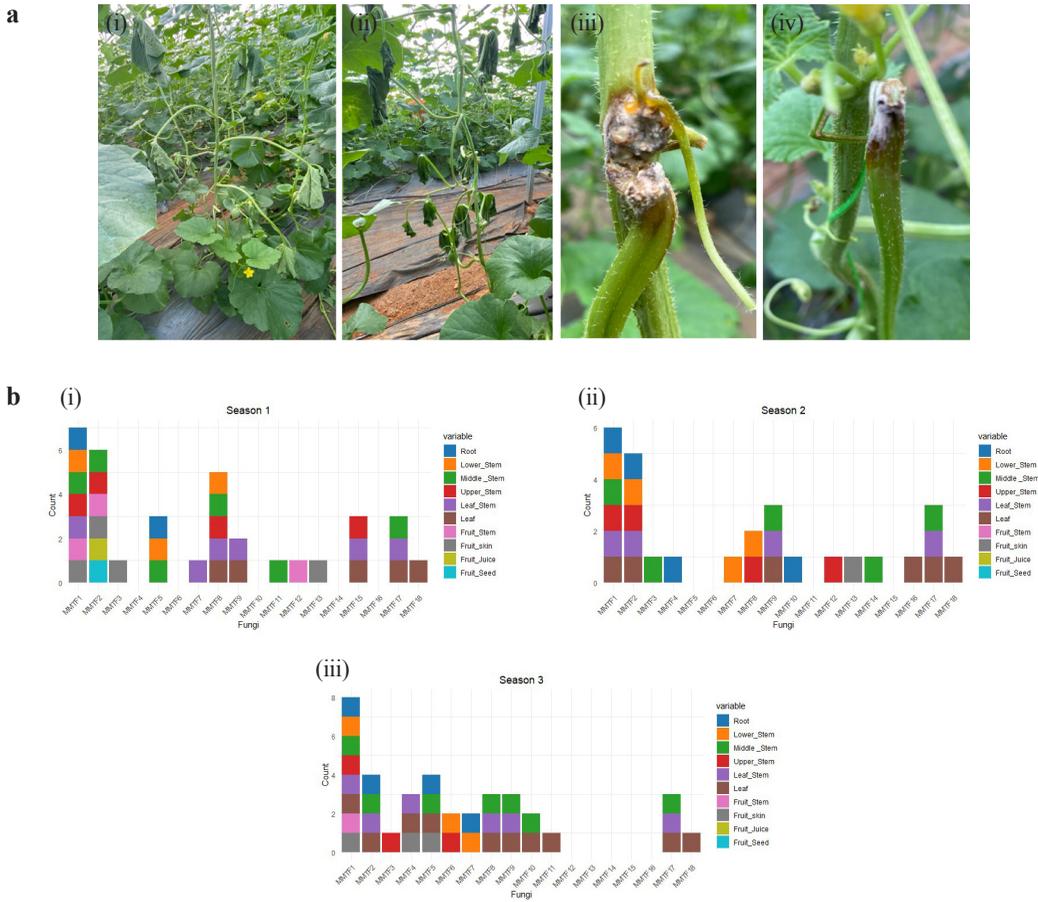


Figure 1: (a) (i) and (ii) plants show symptoms of wilted plant, (iii) and (iv) stem rot symptoms in some of the wilted plants, (b) (i), (ii), and (iii) comparison of 18 fungi found in 10 parts of the plant for three seasons
 Source: Author (2021; 2022; 2023)

regions worldwide, including Brazil, where it is frequently found in rice, as noted by Avila *et al.* (2019). However, knowledge of the toxigenic properties associated with this complex is limited. The complex has also been identified in Tunisian cereals. Because there are more than 30 cryptic phylogenetic species within FIESC, morphological identification becomes a difficult task, as pointed out by Jedidi *et al.* (2021). FIESC’s species composition in China is relatively unknown, even though its presence has been reported in various climatic regions, as highlighted by Lu *et al.* (2021). The precise identification of the species within this complex remains a subject of ongoing exploration.

Based on phylogenetic analysis, the identification of MMTF1 as *F. sulawesiense* provides significant insight into the fungal species responsible for the wilt and stem rot symptoms in melon plants. This identification aligns with the broader context of FIESC, a complex with numerous cryptic species, making its morphological identification challenging.

Morphology Identification

The study investigated the growth and characteristics of *Fusarium sulawesiense* on two types of agar media: Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). The

Table 2: Fungi found in three seasons from the wilted plant of Melon Manis Terengganu plant (October 2020 to May 2021)

Fungi ID	Season 1	Season 2	Season 3
MMTF1	+	+	+
MMTF2	+	+	+
MMTF3	+	+	+
MMTF4	-	+	+
MMTF5	+	-	+
MMTF6	-	-	+
MMTF7	+	+	+
MMTF8	+	+	+
MMTF9	+	+	+
MMTF10	-	+	+
MMTF11	+	-	+
MMTF12	+	+	-
MMTF13	+	+	-
MMTF14	-	+	-
MMTF15	+	-	-
MMTF16	-	+	-
MMTF17	+	+	+
MMTF18	+	+	+

Note: “+” indicates the presence and “-” indicates the absence of fungi in the wilted plants during the respective season.

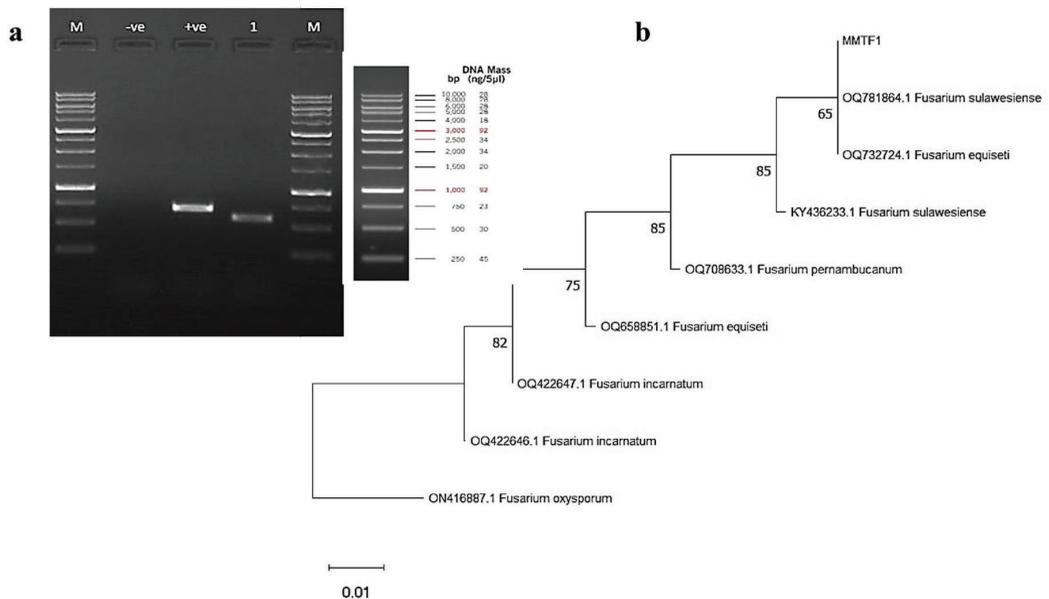


Figure 2: (a) Agarose gel electrophoresis (1%) of PCR product of MMTF1 (M; 1kb DNA ladder, -ve; no template control, +ve; positive control purifies plasmid with ITS region insert 1ng, 1; MMTF1). (b) Phylogenetic tree inferred from MMTF1 using Maximum likelihood (ML), bootstrap values > 65%.

Fusarium oxysporum was used as an outgroup

Source: Author (2023)

results presented in Figure 3 (a) offer a detailed comparison of the cultural characteristics displayed by both agars. The colony grown on PDA showed optimal growth at a temperature of 25°C, with an average growth rate of 4.7 mm to 6.2 mm per day. However, the colony grown on SD exhibited slightly faster growth rates, ranging from 6.4 mm to 8.7 mm per day at the same temperature.

Figure 3 (b) presents the microscopic examination of the mycelium from the *F. sulawesiense* cultures on both PDA and SDA on day four of growth. This detailed analysis provides insights into the structural distinctions between these two cultures. Mycelium grown on SDA appeared to exhibit a higher level of

complexity than mycelium on PDA. Further examination at the microscale revealed that mycelia on SDA displayed signs of shrinkage in certain regions, a phenomenon not observed in the mycelial growth on PDA. These observations emphasise the significance of the choice of agar medium in influencing the morphology and development of *F. sulawesiense*.

It is worth noting that the growth rate and mycelial characteristics of *F. sulawesiense* documented in this study align with findings by Maryani *et al.* (2019), which described *F. sulawesiense* as a relatively fast-growing species with an average daily growth rate falling in the range of 5.2 mm to 6.0 mm/per day. This pace of growth is notably higher when

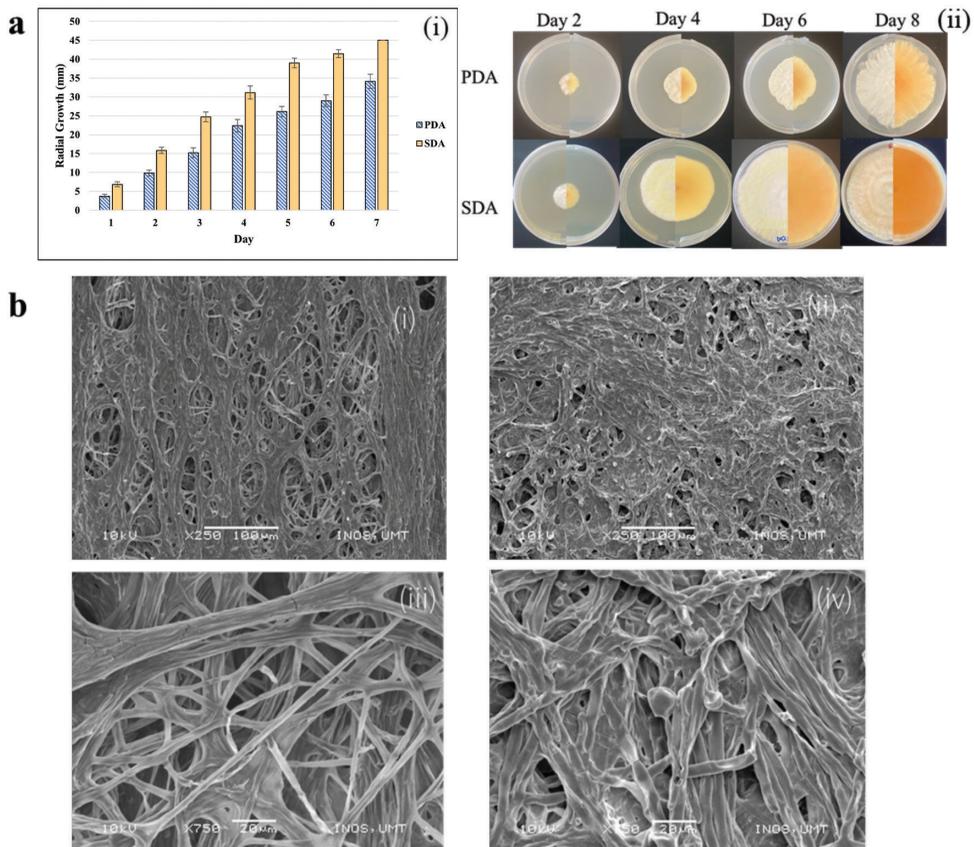


Figure 3: (a) (i) Radial growth of *Fusarium sulawesiense* in two different agar, Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA), (ii) visual comparing *F. sulawesiense* from day two to day eight on PDA and SDA; (b) (i) mycelial on PDA at x250, (ii) mycelial on SDA at x250, (iii) mycelial on PDA at x750, and (iv) mycelial on SDA at x750

Source: Author (2023)

compared to its sister species in the Incarnatum clade, specifically FIESC-34, which displays a comparatively slower average daily growth rate of 1.3 mm to 2.2 mm per day.

Figure 4 provides a detailed depiction of the general morphology of *F. sulawesiense* cultured on PDA. *F. sulawesiense* on PDA was selected, as the fungus on PDA shows clearer morphology than the culture on SDA. A cottony texture with a rosy buff characterises the colony surface and pale orange-like colouration undersurface. Smooth and hyaline hyphae were branched and

septate. The macroconidia produced exhibit a septate structure with a range of four to nine septa, featuring foot-shaped structures at the ends of the septa. The microconidia were oval, kidney, and sickle-shaped with one to four septa. The sporulation is notably abundant, originating from conidiophores borne on the aerial mycelium and sporodochia. These observations provide a comprehensive understanding of the visual and structural attributes of *F. sulawesiense* culture on PDA.

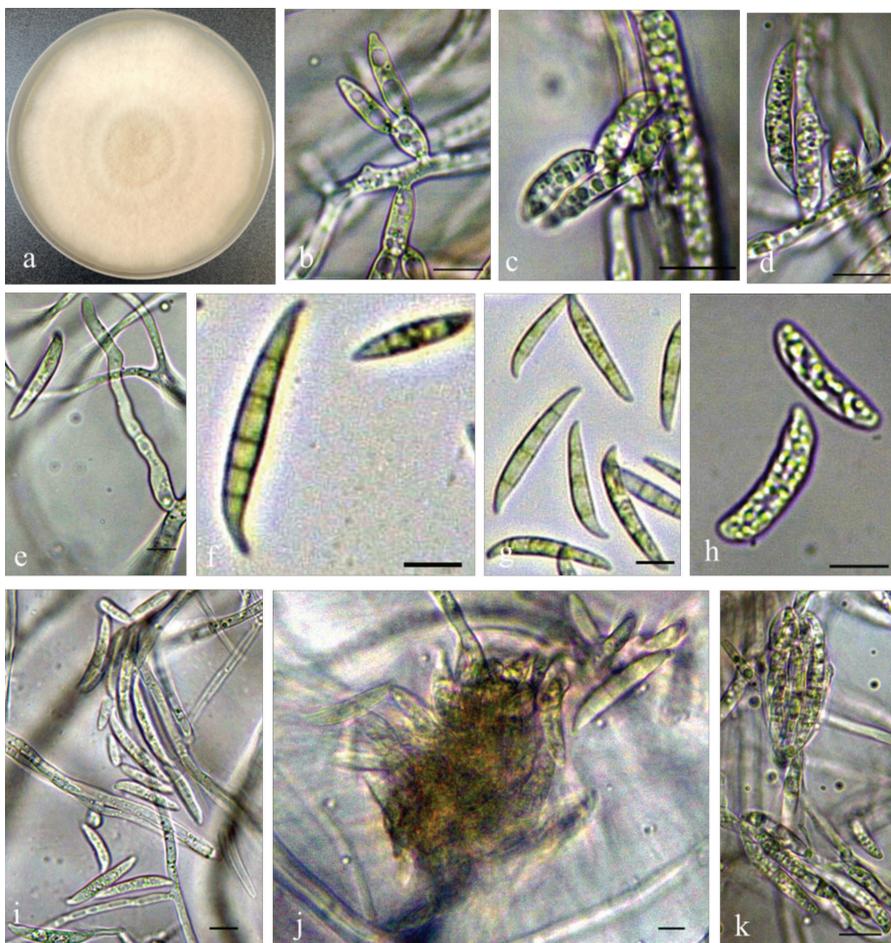


Figure 4: General morphology of *Fusarium Sulawesiense*. (a) Colonies on PDA nine days; (b) polyphialides in aerial mycelial with “rabbit ears” appearance; (c) and (d) mono- and polyphialides from aerial mycelial; (e) aerial conidiophores and phialides; (f) and (g) macroconidia; (h) microconidia; (i), (j), and (k) sporodochia forms on aerial hyphae – Scale bars = 10 um

Source: Author (2023)

Pathogenicity Test

The data presented in Figure 5 provides insight into the pathogenicity of *Fusarium sulawesiense* on the host plant using different inoculation methods. Figure 5 (a) shows that the pathogen had a highly detrimental effect on seed germination. No seed germination occurred from day one to seven, indicating a severe impact with 100% severity. This suggests that the pathogen has the potential to significantly reduce the host plant's ability to reproduce through seeds.

According to Figure 5 (b), when *F. sulawesiense* was sprayed with conidial spores, it resulted in severe symptoms (76% to 100%) within seven days. The leaves turned yellow and wilted, while the stem became split and rotted. On the other hand, Figures 5 (c) and (d) show the results of wound inoculation methods. The wound inoculation by conidial droplet resulted in milder symptoms with a severity of less than 25% by day seven. In contrast, mycelial plug

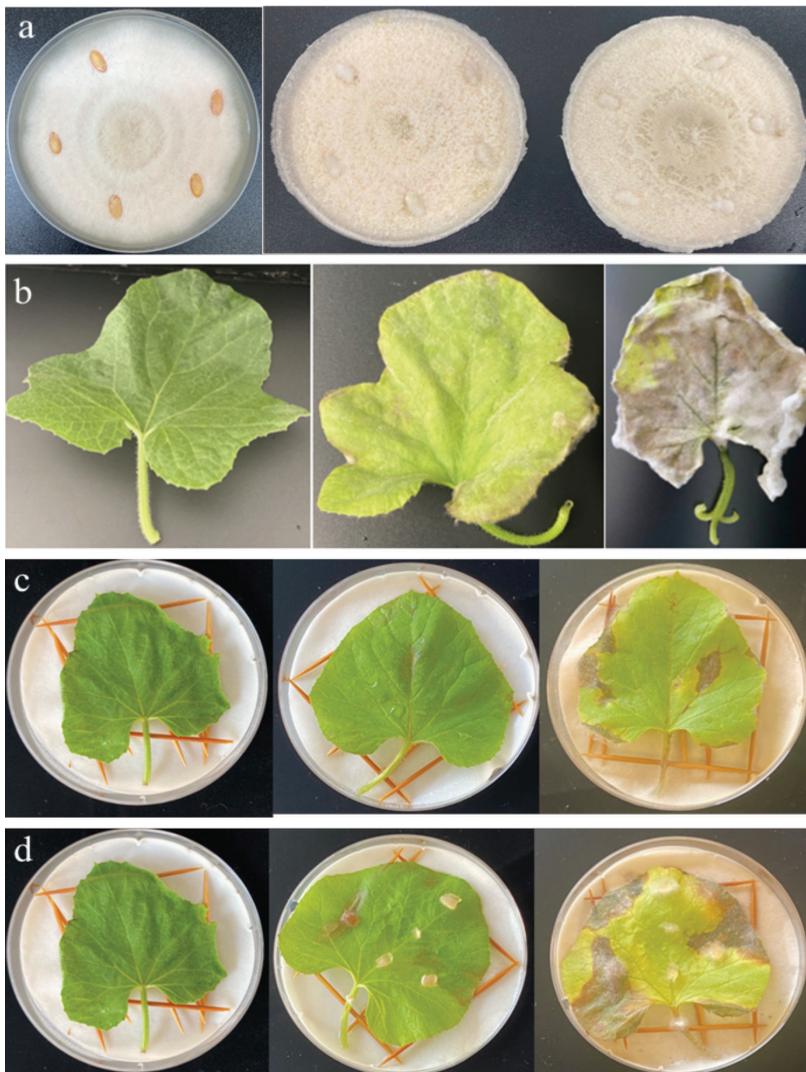


Figure 5: (a) Seed germination inoculation pathogenicity, (b) spraying by conidial spore inoculation method, (c) wound inoculation by conidial droplet, and (d) wound inoculation by mycelial plug
Source: Author (2022)

inoculation caused symptoms with a severity range of 26% to 50%. Wound inoculation with conidial droplets appears less effective in causing disease symptoms than the mycelial plug, which is more effective but still less severe than the spraying method.

Fusarium sp. is a group of harmful fungi that reside in the soil and pose a serious threat to plants. These pathogens invade the root system of plants and cause damage to their vascular structure, causing discolouration from pale yellow to dark red or black, leading to the characteristic symptom of wilting (Sharma *et al.*, 2023). Understanding the behaviour and impact of these fungi is crucial in managing diseases that affect plants and crops.

Disease Incidence and Seasonal Climatic Change (Field Study)

Figure 6 presents critical climate change data and its significant impact on disease as detailed in Table 3. Graph created by the author using Tableau and weather data recorded by Malaysian Meteorological Department (2023). The data reveals that the onset of the monsoon season occurred in November with December experiencing notable environmental shifts. December was characterised by higher evaporation rates exceeding 7 mm, maximum wind speeds exceeding 13 m/s, and substantial rainfall reaching nearly 200 mm daily. Concurrently, December also marked the lowest 24 hour mean temperature and decreased global radiation. These conditions are often associated with elevated humidity levels, which can create a conducive environment for plant diseases.

The MMT plants under observation were sown in mid-November 2021 and harvested in mid-January 2022. The data presented in Table 3 reveals a crucial correlation between environmental conditions and the onset and progression of plant diseases. Notably, the disease affecting the vascular system and resulting in plant wilting was first observed in week three of the plantation on the first week of December 2021 with an initial incidence of 0.56%, indicating that the disease was present

but not widespread. This incidence steadily increased to 61.67% just before the harvesting stage, significantly surpassing the incidence of other diseases. This dramatic increase in disease incidence suggests that the changing climate conditions, particularly the monsoon season, significantly contributed to the spread and severity of the disease.

What is particularly concerning is that this disease ultimately led to the demise of approximately 35.83% of the plants at the time of harvesting during the monsoon season. This statistic underscores the destructive potential of the disease and its ability to cause substantial economic losses to farmers and negatively impact food production.

The results presented in this study emphasise the intricate relationship between climate change, specifically the monsoon season and disease dynamics in melon plants. The increased rainfall, reduced temperature, and higher humidity associated with the monsoon season create an environment conducive to the proliferation of certain plant pathogens. *Fusarium* wilt is a fungal disease that can affect young and mature plants. It can cause damping-off of young seedlings, where the seedlings wilt and die before they have a chance to grow. It can also cause wilting of mature plants, where the leaves turn yellow or brown and the plant ultimately dies (Yang *et al.*, 2023). The disease can be particularly devastating to crops with yield losses of up to 80% occurring under favourable conditions (Karki *et al.*, 2022). These conditions include warm and humid weather, cold spring, and poor soil drainage.

Conclusions

This report presents initial findings of *Fusarium sulawesiense* affecting Melon Manis Terengganu, causing *Fusarium* wilt and stem rot. Research is needed to understand these diseases, considering different climates. Investigating the implications of *F. sulawesiense* is crucial for disease management. Understanding this relationship is critical for farmers. Proactive

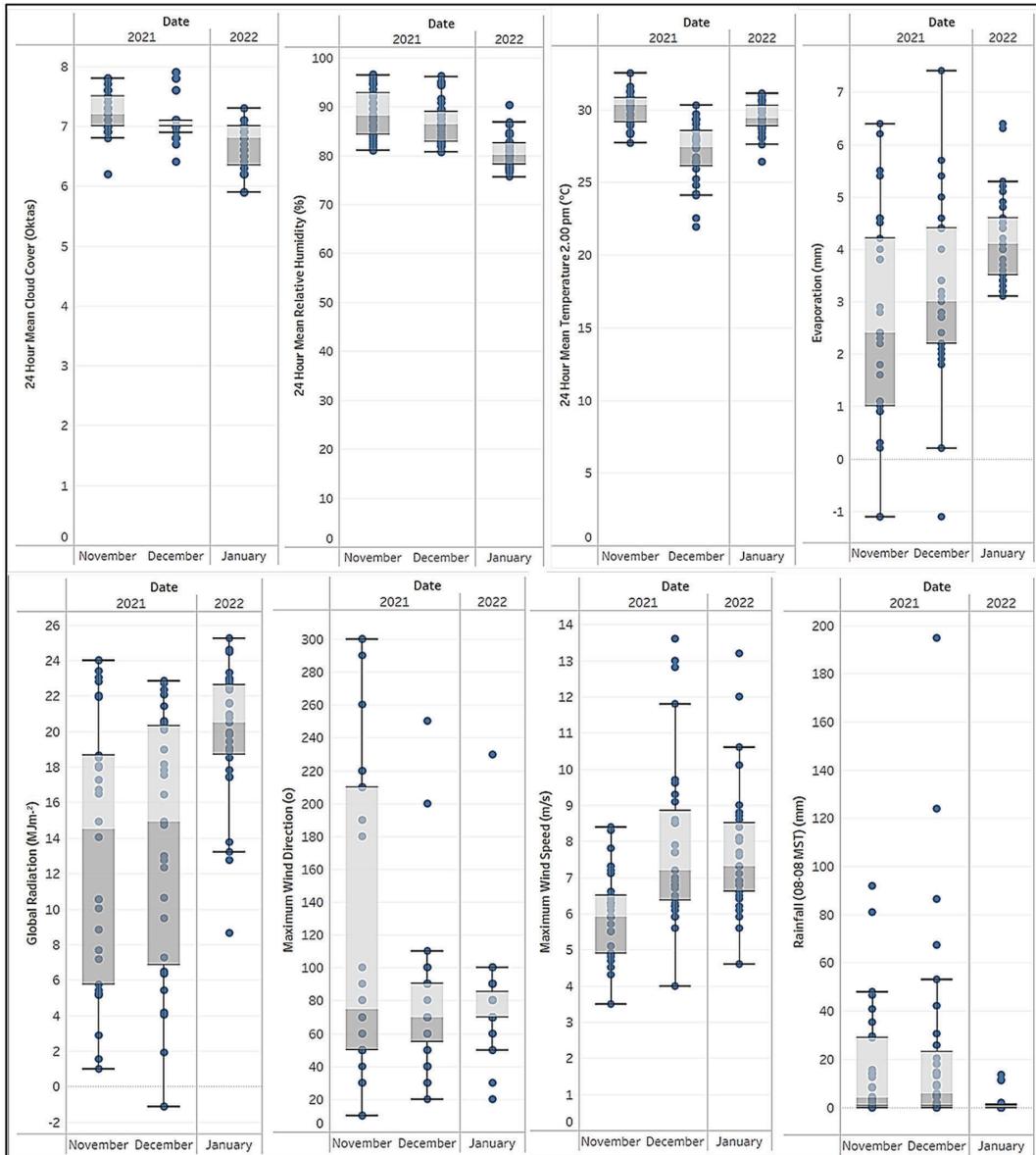


Figure 6: Climate change weather data from November 2021 to January 2022, which includes 24 hour mean cloud cover (oktas), 24 hour mean relative humidity (%), 24 hour mean temperature 2.00 pm (°C), evaporation (mm), global radiation (mJm⁻²), maximum wind direction (°), maximum wind speed (m/s), and rainfall (08-08 MST) (mm)
 Source: Author (2023)

disease management during changing climatic conditions is necessary. Using resistant plant varieties, timely fungicide application and improved irrigation practices can mitigate excess moisture's impact. Ongoing research is important to explore climate change's

influence on plant disease progression. More effective prevention and control strategies can be developed with a deeper understanding, safeguarding crop yields and food security in regions prone to seasonal climatic variations.

Table 3: Disease incidence percentage (%) of Melon Manis Terengganu plantation in monsoon season

Disease	Week							
	1	2	3	4	5	6	7	8
Viruses	0.00	0.00	1.39	4.72	7.22	6.67	6.94	6.11
Disease on vascular and wilted	0.00	0.00	0.56	4.72	19.44	30.00	41.67	61.67
Powdery mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56
Downy mildew	0.00	0.00	0.00	0.56	3.89	4.44	3.61	1.94
Plant dead	0.00	0.00	0.00	0.00	0.00	8.61	18.89	35.83
No disease appeared	100.00	100.00	98.06	91.39	75.56	56.94	35.28	10.28

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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