BUKTI KOREPODENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel : The identification and pathogenicity of Fusarium oxysporum causing acacia seedling wilt disease
Jurnal : Journal of Forestry Research, 33, 711–719 (2021)
Penulis : Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama

NO	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit pertama	26 Februari 2021
2	Bukti konfirmasi review dan hasil review	16 Maret 2021
3	Bukti konfirmasi submit revisi, respon kepada reviewer, dan artikel yang diresubmit	05 April 2021
4	Bukti konfirmasi accepted dan hasil proof corrections	25 April 2021
5	Bukti konfirmasi submit proof corrections, respon kepada editor, dan artikel yang proof corrections	07 Mei 2021
6	Bukti persetujuan terbit jurnal	30 Mei 2021
7	Bukti konfirmasi artikel published online	16 Juli 2021

1.Bukti konfirmasi submit artikel dan artikel yang disubmit pertama (26 Februari 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

A manuscript number has been assigned to The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease

2 messages

Journal of Forestry Research <em@editorialmanager.com> Fri, Feb 26, 2021 at 11:58 AM
Reply-To: Journal of Forestry Research <jfr_nefu@vip.163.com>
To: Ahmad Muslim <a_muslim@unsri.ac.id>

Dear Dr. Muslim,

Your submission entitled "The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease" has been been assigned the following manuscript number: JFR-D-21-00042.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author.
The URL is https://www.editorialmanager.com/jfr/.

Thank you for submitting your work to this journal.

Kind regards,

Lei Yu
Managing Editor
Journal of Forestry Research

_____
In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/jfr/login.asp?a=r). Please contact the publication office if you have any questions.

---

a. muslim unsri <a_muslim@unsri.ac.id> Sat, Feb 27, 2021 at 12:38 PM
To: Journal of Forestry Research <jfr_nefu@vip.163.com>

Dear Prof. Lei Yu

Thank you very much for your response regarding our manuscript submitted in the Journal of Forestry Research.

We really hope our manuscript could be published in the Journal.

We are waiting for a good news of the process review of our manuscript

Thank you

Best Regard
A. Muslim
[Quoted text hidden]

The Identification and Pathogenicity of *Fusarium oxysporum* causing Acacia Seedling Wilt Disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3]Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

Abstract

Fusarium oxysporum is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; consequently, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F. oxysporum* with varying colony size and color pigment were confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the *Fusarium pathogen* was verified as causing vascular disease. Furthermore, Koch's postulate was confirmed by re-isolating the *F.oxysporum* isolate. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

Keyword: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

INTRODUCTION

Acacia mangium is a green leafy plant species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable environmental conditions, this species has a high growth rate, up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies because it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia were 8.67 million hectares, with 81.30% being used for plant cultivation, including *A. mangium.* Furthermore, in 2017, this plant produced the most logs, reaching 77.55% from a total of 40,628.78 m^3 (Statistics Indonesia 2018). The problem faced in cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stage in the process of plants and, if ignored, it will have an impact caused by disease spread in the field.

F. oxysporum is a soil-borne pathogen with a very wide host range and is common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019) including forest and industrial plants (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and generative) and is able to survive on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *Acacia koae* in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F. oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F. oxysporum*, has been found to cause damping-off disease in seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced stage seedlings of *A. mangium* and the disease's pathogenicity.

MATERIAL AND METHODS

Survey and sampling

Soil and diseased plant samples were collected from five commercial company-owned acacia nursery estates with a seedling wilt problem. The diseased plants showed initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Plant samples showing wilting symptoms were collected and stored in a cool box during the process. To determine soil infectivity, acacia seeds were sown using a seedbed, and to accelerate germination, they were soaked in hot water (± 95 ˚C) and left to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate the radicula germination. The germinated seeds were planted on infested field nursery medium. The infected seedlings from the field and the infected soil nursery medium were taken and the pathogen was isolated from the plant tissue.

Fungal isolation

Fungi were isolated from the roots of plants showing the symptoms of seedling wilt, both from the field and the soil infected with pathogens. Then, the root samples were washed under running water, and the surface was sterilized by dipping them in a solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et al. 2012). Then, they were planted in a Petri plate containing agar water with 2% (w/v) agar and 0.1% Streptomycin-sulfate and incubated for 2 × 24 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on the Leslie and Summerell (2006) method. The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed based on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia, macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

A pathogenicity test was carried out on *A. mangium* seedlings 30 days after sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which had previously been sterilized using an autoclave. Fungal isolates were grown in a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a soil medium, while the uninoculated control was watered only with sterile distilled water. Each isolate was inoculated on 10 test plants and the experiment was repeated once. Disease incidence was counted by the number of diseased plants out of the 10 tested plants. The severity of the disease was calculated using a score of 0−4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1−30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3−4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB liquid medium and incubated for 3−4 days at room temperature. Meanwhile, the fungal mycelium was harvested using vacuum filtration and then it was frozen. The DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA) following manufacturer's instructions. Its concentration and quality

were determined by spectrophotometry using a NanoDrop Spectrophotometry ND‐1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at ‐20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ ‐ATGGGTAAGGAAGACAAGAC ‐3′) and EF2 (forward: 5′‐GGAAGTACCAGTGATCATGTT‐3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio‐rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 583 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using the subtree pruning regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

RESULTS AND DISCUSSION

Results

Disease symptoms and wilt incidences

Surveys on seedling wilt were carried out in six acacia nursery locations in commercial companies, with five sites in the Air Sugihan area and one site in Lebong Hitam (Table 1). The incidence of disease varied between locations from as low as 5.6% up to 36.9%. The early symptoms of seedling wilt started from the lower leaves as they turned yellow, and then black, dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves, which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1). Observations were made by planting acacia on used soil medium from the commercial nursery, and the results showed that 56.25% of the seedlings were attacked. In the commercial nursery, this disease was found to attack acacia seedlings at an average age of more than 1 month after germination and before the formation of phyllodes or false leaves.

Morphological characteristic

Fifteen isolates similar to *F. oxysporum* were isolated from the root tissue of diseased plants (Table 2) and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at a temperature of 27 °C. The cultures on the PDA medium produced several color pigments, such as purple, pale purple and reddish-pink, with air hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.38 mm/day). BF06 showed the slowest colony growth rate (7.46 mm/day) compared to the others (Table 3). They all produce many microconidia on the false heads of monophialides. Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm to 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have 0 to 1 septum, but generally 0. The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm

to39.7 ± 5.9 × 3.8 ± 0.4 µm, and have 3 to 7 septa, but generally 3. The chlamydospores produced singly/in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 4). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

The molecular identification achieved by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by making a comparison with those existing in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3−99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.7) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 4). The first clade consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The second clade consisted of single isolates from *A. mangium* (DF11) and *F. oxysporum* (C009W and C010W) from *Cucumis melo*. The third consisted of DF12 and an isolate of *F. oxysporum* from *Musa* sp. The fourth clade consisted of BF05 and EF14, and *F. oxysporum* (CAV189) from *Musa* sp. var. Harare.

Pathogenicity tests

Fusaria, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A. mangium* seedlings. The results showed that the symptom development

198 began with yellowing of the lower leaves or those closest to the base of the stem, these
199 then wilted, curved upward, turned brown to black, became dry, and fell from the plant.
200 This symptom progresses to the top of the plant causing it to wither and die.
201 Furthermore, infected plants show symptoms of stunted plant growth. Initial symptoms
202 appear 7−14 days after inoculation and develop, causing the infected plants to die in an
203 average range of 1 − 10 days. However, some plants experienced complete wilting
204 immediately, without any initial symptoms (Fig. 3). The control plants were healthy
205 and did not show any wilting symptoms. All the isolates were able to infect plants and
206 disease incidence and disease severity were significantly higher compared to the
207 control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease
208 severity and disease progress and their AUDPC values were significantly higher than
209 other isolates and the control (Table 4). Concerning disease incidence, the *Fusarium*
210 isolate presented a high percentage of disease incidence, ranging from 50−100%. This
211 isolate also induced wilting severity ranging from 1.2 − 3.2. The highest disease
212 incidence was caused by isolate BF06 (100%), while the highest disease severity was
213 caused by isolate DF11 (3.2). Based on disease severity, the isolates were grouped into
214 three categories: high (score 2−4), moderate (score 1.2−2), and low score (0−1.2)
215 virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06,
216 and DF11 were included in the high virulence group (2.1−3.2), while FF15, BF09,
217 BF07, AF03, and BF05 were in the moderate (1.6−2.0), and only EF14 was in the low.
218 We observed that this pathogen causes vascular disease, where the pathogen could be
219 isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the
220 pathogens were all re-isolated (100%) from infected plants and it was confirmed that
221 the morphological characters of the pathogen were the same as the inoculated *Fusarium*
222 isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *F. oxysporum* was identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous studies, *F. oxysporum* has been reported to be the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack acacia germinated seeds resulting in damping-off and the identification of this was made solely based on morphological characters (Widyastuti et al. 2013). This research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana.*

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in India with diseases severity of 16.86% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85% severity (Gardner 1980), and damping-off on *A. mangium* in Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots then enter and multiply along the xylem vessel, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

F. oxysporum was determined based on morphological identification, both visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F. oxysporum*, generally exhibiting a short monophialide (Leslie and Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the that most similar to the *F. oxysporum* isolates from *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar and Mohd 2019), *Musa* sp., and *Musa* sp. var. Harare (Fourie et al. 2009).

The field observation of the disease in the commercial nursery showed that about 36.9% of plants died through *Fusarium* wilt. This attack caused huge economic losses because these nurseries provided seedlings for commercial gardening in South Sumatra totaling 1,324,653 hectares. The high disease incidence in the field was probably caused by the use of previous seedlings infested with the pathogens, which led to rapid development and accumulation through the nursery cycle. This is evident in the high disease incidence on soil media. It is likely that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt comes from infested soil, where the pathogen can survive a long time in the soil. The pathogens can also survive in other plant debris (Postic et al. 2012; Altinok 2013)

Conclusion

This study presents the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed

through morphological and *tef1* gene sequencing and Koch's postulate. The main source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from a soil seedling medium infested with pathogens.

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020

REFERENCES

Altinok HH (2013) Fusarium Species Isolated from Common Weeds in Eggplant Fields and Symptomless Hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161: 335–340.

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f . sp . *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150: 609–621.

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4): 166–172.

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. Archives of Phytopathology and Plant Protection 43(2): 174–190.

Edel-Hermann V, Lecomte C (2019) Current Status of *Fusarium oxysporum* Formae Speciales and Races. J Phytopathol 109: 512–530.

Fourie G, Steenkamp ET, Gordon TR, Viljoen A (2009) Evolutionary relationships among the *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups. Appl Environ Microbiol 75(14): 4770–4781.

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp.

koae, f. sp. nov. J Phytopathol 70(7): 594–597.

Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev Phytopathol 55: 23–39.

Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd - A Fast Growing Tree for Tropical Plantation. J For Sci 29(1): 1–14.

Jiménez-Díaz RM, Castillo P, Jiménez-Gasco MdelM, Landa BB, Navas-Cortés JA (2015) Fusarium wilt of chickpeas: Biology, ecology and management. Crop Protection 73: 16–27.

Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused by *Fusarium oxysporum*. Journal of Tropical Forest Science 16(4): 453–462.

Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3): 442–454.

Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni M (2016) *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla. Plant Pathol 65(4): 612–625.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870–1874.

Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell Publishing, Oxford.

Luo X, Yu C (2020) First report of damping‐off disease caused by *Fusarium oxysporum* in *Pinus massoniana* in China. Journal Plant Diseases and Protection

326 127: 401–409.

327 Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium*
328 sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management
329 strategies. Journal of Applied Research on Medicinal and Aromatic Plants 100244.

330 Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University
331 Press, New York.

332 O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins
333 of the fungus causing Panama disease of banana : Concordant evidence from
334 nuclear and mitochondrial gene genealogies. Proc Natl Sci USA 95: 2044–2049.

335 Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt, focusing
336 on bananas. Appl Soil Ecol 132: 20–33.

337 Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of
338 Fusarium Species Isolated from Weeds and Plant Debris in Croatia. J Phytopathol
339 160: 76–81.

340 Statistics Indonesia (2018) Timber Culture Establishment. Statistics Indonesia, Jakarta.

341 Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as
342 a causal agent of mung bean wilt in China. Crop Protection 117: 77–85.

343 Suwandi, Akino S, Kondo N (2012) Common Spear Rot of Oil Palm in Indonesia
344 Common Spear Rot of Oil Palm in Indonesia. Plant Dis 96(4): 537-543.

345 Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-
346 Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper
347 in Mexico. Can J Plant Pathol 40(2): 238–247.

348 Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum*
349 fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2):
350 110–118.

Table 1 Disease incidence in the commercial nursery fields of *A. mangium* forestry in South Sumatra

No.	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1.	Air Sugihan A	500	34	6.8
2.	Air Sugihan B	720	70	13.5
3.	Air Sugihan C	720	131	18.2
4.	Air Sugihan D	720	266	36.9
5.	Lebong Hitam	604	36	5.6
6.	Air Sugihan F	720	139	19.3

354 Table 2 Isolate origin used for pathogenicity test.

Location	Host	Isolate	Number of isolates
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

355

356 Table 3 Cultural characteristics of *Fusarium oxysporum* obtained from Acacia seedling wilt

357 disease.

Isolate	Colony color	Growth rate (mm/day)
AF01	Top: White to dark pink Bottom: dark pink	11.2 ± 0.6e
AF02	Top: White to pale violet Bottom: Pale violet	10.7 ± 0.6d
AF03	Top: white Bottom: pale violet	10.7 ± 0.5d
AF04	Top: white Bottom: Pale violet	11.1 ± 0.9e
BF05	Top: White to pale violet Bottom: Violet	11.4 ± 0.8e
BF06	Top: White to pale violet Bottom: Violet	7.5 ± 0.7a
BF07	Top: White to dark pink Bottom: Dark pink	7.7 ± 0.6ab
BF08	Top: White to pale violet Bottom: Pale violet	9.0 ± 0.2abc
BF09	Top: White Bottom: Violet to pale violet	8.1 ± 0.6abc
CF10	Top: White to dark pink Bottom: dark pink	9.0 ±1.5bc
DF11	Top: White to dark pink Bottom: dark pink	9.5 ± 0.4cd
DF12	Top: white to pale violet Bottom: pale violet	8.4 ± 0.5abc
DF13	Top: white to pale violet Bottom: pale violet	8.2 ± 0.4abc
EF14	Top: white Bottom: pale violet	9.3 ± 0.8cd
FF15	Top: White Bottom: Violet	11.4 ± 2.1e
LSD 0.05		2.04

358 The numbers followed by the same letter in the column are not significantly different in the LSD

359 test at 5%.

360 Table 4 Characteristics of the microscopic structure of *Fusarium oxysporum* isolated from infected plants.

Isolate	Microconidia (μm)		Macroconidia (μm)		Chlamydospore (μm)	
	Characteristics	**Size (L × W)**	**Characteristics**	**Size (L × W)**	**Characteristics**	**Size (D)**
AF01	Ellipse−allantoid, 0−1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3−4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Oval−reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3−4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Ellipse−oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3−4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Ellipse−reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3−4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Ellipse−allantoid, 0−1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3−4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Allantoid shaped, 0−2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3−4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Oval−allantoid shaped, 0−2 septa, mostly 0−1septum	7.6 ± 1.8 × 3.5 ± 0.3	3−4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Allantoid shaped, 0−2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3−4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Oval−ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3−4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Ellipse−allantoid, 0−1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3−7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Ellipse−allantoid, 0−1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3−4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Ellipse−allantoid shaped, 0−1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3−4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Ellipse−allantoid, 0−1 septum, mostly 0 septum	10. 3 ± 2.7 × 2.5 ± 0.3	3−4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7
EF14	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3−4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Oval−ellipse, 0−1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3−4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
Mean		8.1 ± 1.7 × 2.8 ± 0.3		37.5 ± 5.6 × 3.9 ± 0.4		7.7 ± 1.0

361

362 Table 5 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings

363 inoculated with *F. oxysporum*.

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 ab	31.0 ab
AF02	80	2.4 a	46.4 a
AF03	80	1.9 ab	32.6 ab
AF04	80	2.5 a	33.9 ab
BF05	50	2.0 ab	45.5 ab
BF06	100	3.1 a	59.5 a
BF07	60	1.9 ab	32.6 ab
BF08	70	2.1 ab	37.5 ab
BF09	70	1.6 ab	23.6 ab
CF10	90	2.7 a	37.9 a
DF11	90	3.2 a	55.7 a
DF12	80	2.8 a	49.8 a
DF13	80	2.9 a	54.7 a
EF14	60	1.2 ab	15.3 ab
FF15	50	1.6 ab	32.0 ab
Control	0	0 b	0.0 b
P-Value		0.0013	0.0007

364 The numbers followed by the same letter in the column are not significantly different in the LSD

365 test at 5%.

Fig. 1 Wilt symptoms in the nursery (a) yellowing leaves (b) dry leaves (c).

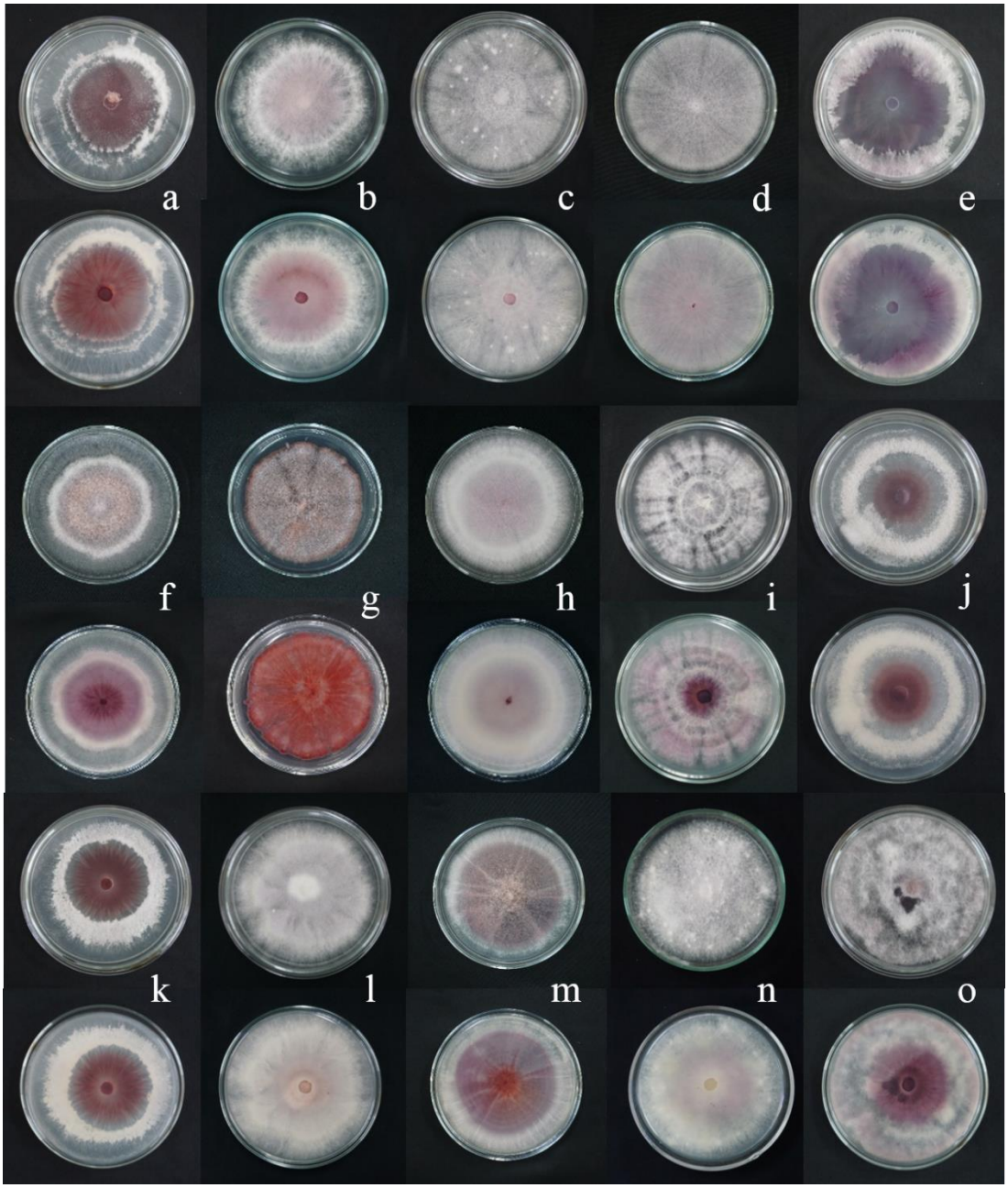
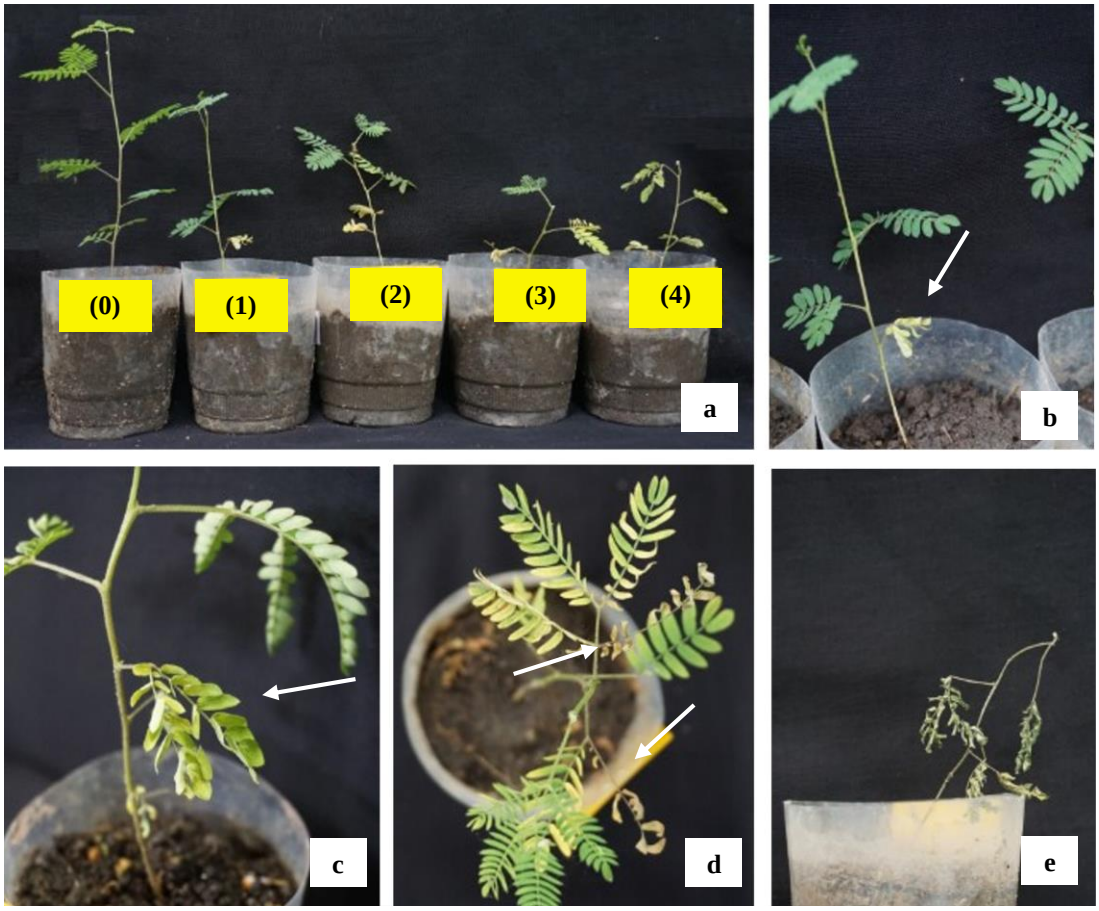


Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f), BF07 (g), BF08 (h), BF09 (i), CF10 (j), DF11 (k), DF12 (l), DF13 (m), EF14 (n), and FF15 (o). Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).

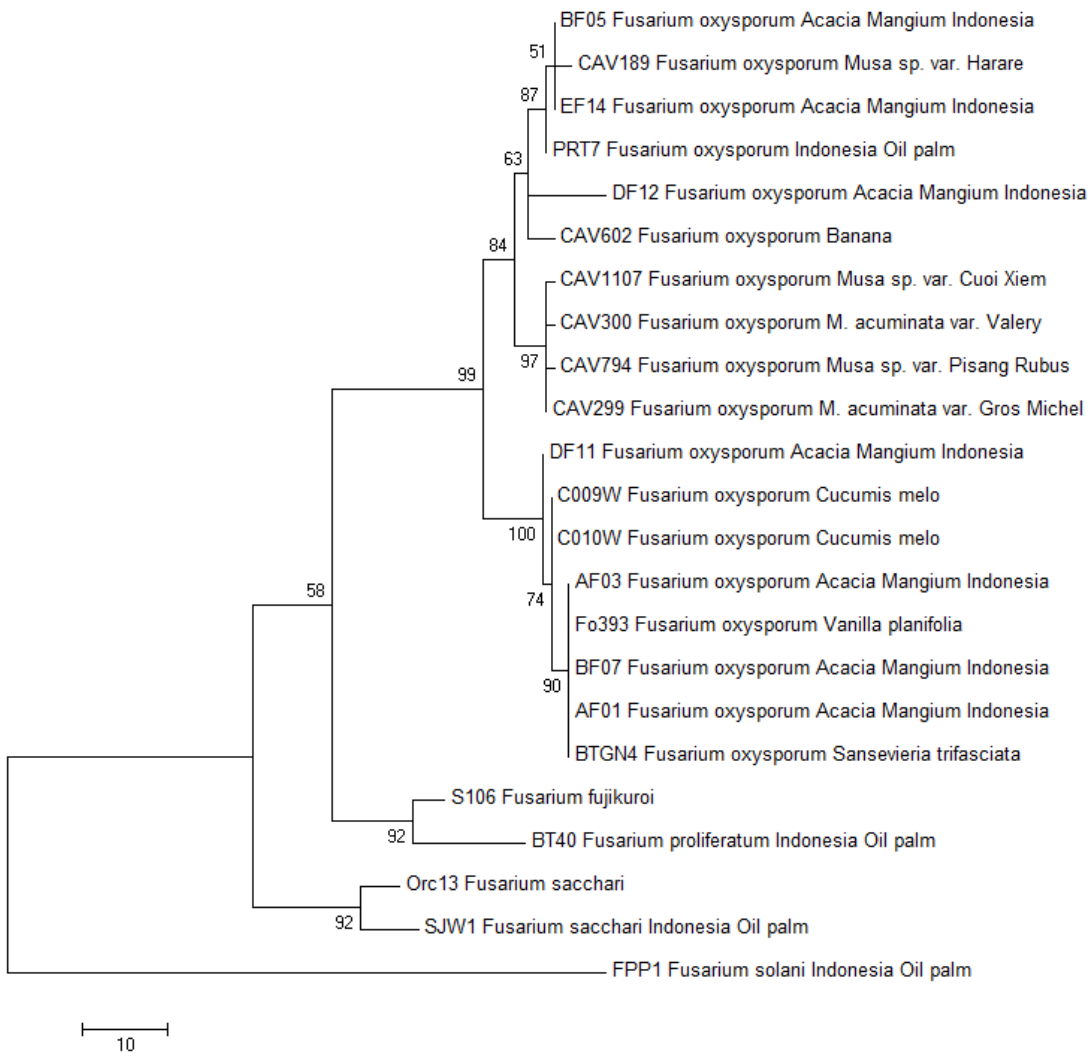


373

374 Fig. 3 Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial

375 symptoms: from lowest leaf, yellowing leaves (b). Advanced symptoms: curved leaves, dry leaves,

376 falling leaves (c,d). and dead plant (e).



377

378 Fig. 4 One out of the six most parsimonious trees showing the genetic relatedness, represented by
379 the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold), closely related
380 *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host
381 species and countries of origin are given with the representative isolates. A bootstrap test with
382 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The
383 genetic distance is indicated by the scale bar.

2. Bukti konfirmasi review dan hasil review (16 Maret 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Your Submission

1 message

Journal of Forestry Research <em@editorialmanager.com> Tue, Mar 16, 2021 at 11:16 AM
Reply-To: Journal of Forestry Research <jfr_nefu@vip.163.com>
To: Ahmad Muslim <a_muslim@unsri.ac.id>

CC: "Soleha Soleha" soleha@student.pps.unsri.ac.id, "Suwandi Suwandi" suwandi@fp.unsri.ac.id, "Sabaruddin Kadir" sabar@pps.unsri.ac.id, "Rahmat Pratama" rahmatpratama@pps.unsri.ac.id

Ref.: Ms. No. JFR-D-21-00042
The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease
Journal of Forestry Research

Dear Dr. Muslim,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. After you revise it well, it will be accepted. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.

Your revision is due by Apr 15, 2021.

To submit a revision, go to https://www.editorialmanager.com/jfr/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Editor
Journal of Forestry Research

Reviewers' comments:

Reviewer #1: Title of Manuscript: The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease

In general, the manuscript is well prepared and written and is reporting Fusarium oxysporum associated with the wilt disease of acacia seedling in South Sumatra, Indonesia. They conducted quite an intensive sampling of acacia seedling from five (six?) areas of South Sumatra. Phylogenetic analyses performed in the study would also appropriate. Then, the data themselves are quite interesting for considering and controlling the disease. It is basically worth publishing also for the future studies.

Abstract: Improve the abstract. There should be brief methodology and the objectives of the study should be clear.

Introduction: information about the phylogeny and taxonomy of Fusarium, in particular of species isolated from acacia seedlings, is missing. It is of main importance to know if TEF alone is really sufficient to distinguish between species. In fungi, it is often impossible to determine a species based on the sequencing of a single gene. If the situation is different for Fusarium, this should be clearly demonstrated. The taxonomic level (Genus, complex of species, species) identified by sequencing only the TEF gene, should in particular be clarified.

Materials and Methods:

Line 62 : "…collected from five commercial company….", however in Results and Discussions (line 149): "…. were carried out in six acacia nursery locations in commercial companies." Which one is correct?

Results and Discussions:

Line 181: Seven isolates were selected for molecular analysis. There is no obvious reasoning why were the seven

isolates selected among 15 isolates. It should be described in methodology.

Lines 201-203: "Initial symptoms appear 7–14 days after inoculation and develop, causing the infected plants to die in an average range of 1–10 days". The sentence is difficult to be understood by the reader. From what stage plants died?

Lines 239-240: "…….and damping-off on A. mangium in Indonesia with unknown disease severity (Widyastuti et al. 2013)". What part of Indonesia should be noticed because the authors declared the first report of F oxysporum in South Sumatra

Lines 351-352 (Table 1): … A. mangium should be written Acacia mangium

Lines 351-352 (Table 1): There are some mistakes on the calculation of disease incidence on the location of Air Sugihan B and Lebong Hitam. Please recalculate them.

Lines 356-357 (Table 3): Colony color can be inserted in Table 4 and the title of Table 4 will be revised by adding: macroscopic.

Lines 356-357 (Table 3): Growth rate of the isolates can be illustrated as bar chart.

Reviewer #3: First of all, I will say that this document was extremely well prepared. The pathology problem being explored is extremely important and all of the methodology that was used was well explained and appropriate. There is no doubt that this article should be published.

I did, however, note that in some cases slightly different word choices might make some of the text a little easier to understand and therefore took the liberty of making changes where I thought they would be appropriate. These suggested changes are shown in the attached document.

Oh yes, there is one other thing. The last part of line 128 should read .....and EF2 (reverse: 5'-GGAAGTACCAGTGATCATGTT)-3') rather than ....and EF2(forward: 5'-GGAAGTACCAGTGATCATGTT)-3').

_____

The Identification and Pathogenicity of *Fusarium oxysporum* causing Acacia Seedling Wilt Disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3]Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

Abstract

Fusarium oxysporum is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; consequently, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F. oxysporum* with varying colony size and color pigment were confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the *Fusarium pathogen* was verified as causing vascular disease. Furthermore, Koch's postulate was confirmed by re-isolating the *F.oxysporum* isolate. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

Commented [A1]: The result should be described in abstract as conclusion.

Commented [A2]: Not italics : pathogen

Commented [A3]: isolates

Commented [A4]: There is no information neither purpose nor methodology of this statement in abstract.

Keyword: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

INTRODUCTION

Acacia mangium is a green leafy plant species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable environmental conditions, this species has a high growth rate, up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies because it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia were 8.67 million hectares, with 81.30% being used for plant cultivation, including *A. mangium*. Furthermore, in 2017, this plant produced the most logs, reaching 77.55% from a total of 40,628.78 m^3 (Statistics Indonesia 2018). The problem faced in cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stage in the process of plants and, if ignored, it will have an impact caused by disease spread in the field.

F. oxysporum is a soil-borne pathogen with a very wide host range and is common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019) including forest and industrial plants (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and generative) and is able to survive on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *Acacia koae* in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F. oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F. oxysporum*, has been found to cause damping-off disease in seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced stage seedlings of *A. mangium* and the disease's pathogenicity.

MATERIAL AND METHODS

Survey and sampling

Soil and diseased plant samples were collected from five commercial company-owned acacia nursery estates with a seedling wilt problem. The diseased plants showed initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Plant samples showing wilting symptoms were collected and stored in a cool box during the process. To determine soil infectivity, acacia seeds were sown using a seedbed, and to accelerate germination, they were soaked in hot water (± 95 ˚C) and left to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate the radicula germination. The germinated seeds were planted on infested field nursery medium. The infected seedlings from the field and the infected soil nursery medium were taken and the pathogen was isolated from the plant tissue.

Commented [A5]: Five or six? See line 149.

Fungal isolation

Fungi were isolated from the roots of plants showing the symptoms of seedling wilt, both from the field and the soil infected with pathogens. Then, the root samples were washed under running water, and the surface was sterilized by dipping them in a solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et al. 2012). Then, they were planted in a Petri plate containing agar water with 2% (w/v) agar and 0.1% Streptomycin-sulfate and incubated for 2 × 24 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on the Leslie and Summerell (2006) method. The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed based on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia, macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

A pathogenicity test was carried out on *A. mangium* seedlings 30 days after sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which had previously been sterilized using an autoclave. Fungal isolates were grown in a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a soil medium, while the uninoculated control was watered only with sterile distilled water. Each isolate was inoculated on 10 test plants and the experiment was repeated once. Disease incidence was counted by the number of diseased plants out of the 10 tested plants. The severity of the disease was calculated using a score of 0−4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1−30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3−4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB liquid medium and incubated for 3−4 days at room temperature. Meanwhile, the fungal mycelium was harvested using vacuum filtration and then it was frozen. The DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA) following manufacturer's instructions. Its concentration and quality

were determined by spectrophotometry using a NanoDrop Spectrophotometry ND‐1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at‐20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ ‐ATGGGTAAGGAAGACAAGAC ‐3′) and EF2 (forward: 5′‐GGAAGTACCAGTGATCATGTT‐3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio‐rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 583 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using the subtree pruning regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

RESULTS AND DISCUSSION

Results

Disease symptoms and wilt incidences

Surveys on seedling wilt were carried out in six acacia nursery locations in commercial companies, with five sites in the Air Sugihan area and one site in Lebong Hitam (Table 1). The incidence of disease varied between locations from as low as 5.6% up to 36.9%. The early symptoms of seedling wilt started from the lower leaves as they turned yellow, and then black, dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves, which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1). Observations were made by planting acacia on used soil medium from the commercial nursery, and the results showed that 56.25% of the seedlings were attacked. In the commercial nursery, this disease was found to attack acacia seedlings at an average age of more than 1 month after germination and before the formation of phyllodes or false leaves.

Commented [A6]: Six or five? See lines 62

Morphological characteristic

Fifteen isolates similar to *F. oxysporum* were isolated from the root tissue of diseased plants (Table 2) and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at a temperature of 27 °C. The cultures on the PDA medium produced several color pigments, such as purple, pale purple and reddish-pink, with air hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.38 mm/day). BF06 showed the slowest colony growth rate (7.46 mm/day) compared to the others (Table 3). They all produce many microconidia on the false heads of monophialides. Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm to 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have 0 to 1 septum, but generally 0. The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm

to39.7 ± 5.9 × 3.8 ± 0.4 µm, and have 3 to 7 septa, but generally 3. The chlamydospores produced singly/in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 4). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

The molecular identification achieved by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by making a comparison with those existing in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3−99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.7) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 4). The first clade consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The second clade consisted of single isolates from *A. mangium* (DF11) and *F. oxysporum* (C009W and C010W) from *Cucumis melo*. The third consisted of DF12 and an isolate of *F. oxysporum* from *Musa* sp. The fourth clade consisted of BF05 and EF14, and *F. oxysporum* (CAV189) from *Musa* sp. var. Harare.

Commented [A7]: Why the seven isolates are selected should be described in methodology.

Pathogenicity tests

Fusaria, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A. mangium* seedlings. The results showed that the symptom development

198 began with yellowing of the lower leaves or those closest to the base of the stem, these
199 then wilted, curved upward, turned brown to black, became dry, and fell from the plant.
200 This symptom progresses to the top of the plant causing it to wither and die.
201 Furthermore, infected plants show symptoms of stunted plant growth. Initial symptoms
202 appear 7−14 days after inoculation and develop, causing the infected plants to die in an
203 average range of 1 − 10 days. However, some plants experienced complete wilting
204 immediately, without any initial symptoms (Fig. 3). The control plants were healthy
205 and did not show any wilting symptoms. All the isolates were able to infect plants and
206 disease incidence and disease severity were significantly higher compared to the
207 control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease
208 severity and disease progress and their AUDPC values were significantly higher than
209 other isolates and the control (Table 4). Concerning disease incidence, the *Fusarium*
210 isolate presented a high percentage of disease incidence, ranging from 50−100%. This
211 isolate also induced wilting severity ranging from 1.2 − 3.2. The highest disease
212 incidence was caused by isolate BF06 (100%), while the highest disease severity was
213 caused by isolate DF11 (3.2). Based on disease severity, the isolates were grouped into
214 three categories: high (score 2−4), moderate (score 1.2−2), and low score (0−1.2)
215 virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06,
216 and DF11 were included in the high virulence group (2.1−3.2), while FF15, BF09,
217 BF07, AF03, and BF05 were in the moderate (1.6−2.0), and only EF14 was in the low.
218 We observed that this pathogen causes vascular disease, where the pathogen could be
219 isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the
220 pathogens were all re-isolated (100%) from infected plants and it was confirmed that
221 the morphological characters of the pathogen were the same as the inoculated *Fusarium*
222 isolates. The pathogen was not isolated from the uninoculated control plants.

Commented [A8]: From what stage?

223

224 **Discussion**

225 This research reports that *F. oxysporum* was identified for the first time as a
226 causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According
227 to previous studies, *F. oxysporum* has been reported to be the cause of seedling wilt in
228 *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In
229 Indonesia, this pathogen was reported to attack acacia germinated seeds resulting in
230 damping-off and the identification of this was made solely based on morphological
231 characters (Widyastuti et al. 2013). This research confirms that *F. oxysporum*,
232 identified by morphological and molecular methods, causes vascular wilt disease in
233 advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that
234 *F. oxysporum* causes damping-off on *Pinus massoniana.*

235 The pathogenicity test confirmed that all the isolates were able to develop wilt
236 symptoms with different disease incidence and severity, some reaching 90−100% and
237 scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt
238 diseases on *A. nilotica* in India with diseases severity of 16.86% (Kapoor et al. 2004),
239 on *A. koa* in Hawaii with 85% severity (Gardner 1980), and damping-off on *A. mangium*
240 in Indonesia with unknown disease severity (Widyastuti et al. 2013).

Commented [A9]: What part of Indonesia should be noticed because the authors declare the first report of F oxysporum in South Sumatra.

241 The results here have also shown that *F. oxysporum* is a pathogen causing
242 vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots.
243 The pathogens infect the roots then enter and multiply along the xylem vessel, and are
244 translocated to the shoots through water movement. Browning in the xylem tissue,
245 which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in
246 the inoculated plants.

247 *F. oxysporum* was determined based on morphological identification, both
248 visually and microscopically. Visually, the fungus produces pale purple to pale pink
249 pigments on a PDA medium. Microscopically, the shape of its reproductive structure is
250 the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and
251 Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-
252 causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which
253 causes acacia seedlings to wilt, is the that most similar to the *F. oxysporum* isolates
254 from *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo*
255 (Bakar and Mohd 2019), *Musa* sp., and *Musa* sp. var. Harare (Fourie et al. 2009).
256 The field observation of the disease in the commercial nursery showed that
257 about 36.9% of plants died through *Fusarium* wilt. This attack caused huge economic
258 losses because these nurseries provided seedlings for commercial gardening in South
259 Sumatra totaling 1,324,653 hectares. The high disease incidence in the field was
260 probably caused by the use of previous seedlings infested with the pathogens, which
261 led to rapid development and accumulation through the nursery cycle. This is evident
262 in the high disease incidence on soil media. It is likely that clonal dispersion occurred
263 via the nursery medium to the commercial field nursery as shown by isolates within the
264 first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al.
265 (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt
266 comes from infested soil, where the pathogen can survive a long time in the soil. The
267 pathogens can also survive in other plant debris (Postic et al. 2012; Altinok 2013)
268
269 **Conclusion**
270 This study presents the first report of *F. oxysporum* as a causal agent of *A.*
271 *mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed

through morphological and *tef1* gene sequencing and Koch's postulate. The main source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from a soil seedling medium infested with pathogens.

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020

REFERENCES

Altinok HH (2013) Fusarium Species Isolated from Common Weeds in Eggplant Fields and Symptomless Hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161: 335–340.

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f . sp . *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150: 609–621.

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4): 166–172.

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. Archives of Phytopathology and Plant Protection 43(2): 174–190.

Edel-Hermann V, Lecomte C (2019) Current Status of *Fusarium oxysporum* Formae Speciales and Races. J Phytopathol 109: 512–530.

Fourie G, Steenkamp ET, Gordon TR, Viljoen A (2009) Evolutionary relationships among the *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups. Appl Environ Microbiol 75(14): 4770–4781.

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp.

304 *koae*, f. sp. nov. J Phytopathol 70(7): 594–597.

305 Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev
306 Phytopathol 55: 23–39.

307 Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd - A Fast Growing Tree
308 for Tropical Plantation. J For Sci 29(1): 1–14.

309 Jiménez-Díaz RM, Castillo P, Jiménez-Gasco MdelM, Landa BB, Navas-Cortés JA
310 (2015) Fusarium wilt of chickpeas: Biology, ecology and management. Crop
311 Protection 73: 16–27.

312 Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused
313 by *Fusarium oxysporum*. Journal of Tropical Forest Science 16(4): 453–462.

314 Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of
315 Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3):
316 442–454.

317 Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni
318 M (2016) *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root
319 and stem rot of vanilla. Plant Pathol 65(4): 612–625.

320 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics
321 Analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870–1874.

322 Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell
323 Publishing, Oxford.

324 Luo X, Yu C (2020) First report of damping-off disease caused by *Fusarium oxysporum*
325 in *Pinus massoniana* in China. Journal Plant Diseases and Protection 127: 401–

409.

Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium* sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. Journal of Applied Research on Medicinal and Aromatic Plants 100244.

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana : Concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Sci USA 95: 2044–2049.

Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt, focusing on bananas. Appl Soil Ecol 132: 20–33.

Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium Species Isolated from Weeds and Plant Debris in Croatia. J Phytopathol 160: 76–81.

Statistics Indonesia (2018) Timber Culture Establishment. Statistics Indonesia, Jakarta.

Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Protection 117: 77–85.

Suwandi, Akino S, Kondo N (2012) Common Spear Rot of Oil Palm in Indonesia Common Spear Rot of Oil Palm in Indonesia. Plant Dis 96(4): 537-543.

Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant Pathol 40(2): 238–247.

348 Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum*
349 fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2):
350 110–118.

Journal of Forestry Research

The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease

--Manuscript Draft--

Manuscript Number: JFR-D-21-00042

Full Title: The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease

Article Type: Original Article

Section/Category: Forest entomology and pathology

Keywords: Acacia mangium; Fusarium oxysporum; Seedling wilt; pathogenicity

Manuscript Region of Origin: INDONESIA

Abstract: Fusarium oxysporum is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; consequently, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in Acacia mangium and its pathogenicity. To achieve this, 15 isolates of F. oxysporum with varying colony size and color pigment were confirmed by observing the morphological characters and elongation factor 1-α (tef1-α) gene sequences. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the Fusarium pathogen was verified as causing vascular disease. Furthermore, Koch's postulate was confirmed by re-isolating the F.oxysporum isolate. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

see next page

The Identification and Pathogenicity of *Fusarium oxysporum* causing Acacia Seedling Wilt Disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3]Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

Abstract

Fusarium oxysporum is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; consequently, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F. oxysporum* with varying colony size and color pigment were confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the *Fusarium pathogen* was verified as causing vascular disease. Furthermore, Koch's postulates was confirmed by re-isolating the *F.oxysporum* isolate. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

Keyword: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

INTRODUCTION

Acacia mangium is a green leafy plant species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable environmental conditions, this species ~~has a high growth rate~~ can grow, up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies because it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia were 8.67 million hectares, with 81.30% being used for plant cultivation, including *A. mangium*. Furthermore, in 2017, this plant produced the most logs, reaching 77.55% from a total of 40,628.78 m^3 (Statistics Indonesia 2018). The main problem faced in cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stage of plant growth ~~in the process of plants~~ and, if ignored, it can spread widely ~~will have an impact caused by disease spread in the field~~ in a plantation.

F. oxysporum is a soil-borne pathogen with a very wide host range and is common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019) including forest and industrial ~~plants~~ plantations (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and ~~generative~~ reproductive) and is able to survive on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *Acacia ~~koa~~* in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F. oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F. oxysporum*, has been found to cause damping-off disease in seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced stage seedlings of *A. mangium* and the disease's pathogenicity.

MATERIAL AND METHODS

Survey and sampling

Soil and diseased plant samples were collected from five commercial company-owned acacia nursery estates with a seedling wilt problem. The diseased plants showed initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Plant samples showing wilting symptoms were collected and stored in a cool box during the process. To determine soil infectivity, acacia seeds were sown using a seedbed, and to accelerate germination, they were soaked in hot water (± 95 °C) and left to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate the ~~radicula germination~~ radical development. The germinated seeds were planted on infested field nursery medium. The infected seedlings from the field and the ~~infected~~ infested soil nursery medium were ~~taken~~ sampled and the pathogen was also isolated from the plant tissue.

Fungal isolation

Fungi were isolated from the roots of plants growing in the field that showed wilt symptoms and also from ~~showing the symptoms of seedling wilt, both from the field and the~~ soil infested with pathogens. Then, the root samples were washed under running water, and the surface was sterilized by dipping them in a solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et al. 2012). Then, they were planted in a Petri plate containing agar water with 2% (w/v) agar and 0.1% Streptomycin-sulfate and incubated for ~~2 × 24~~ 48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on the Leslie and Summerell (2006) method. The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed based on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia, macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

A pathogenicity test was carried out on *A. mangium* seedlings 30 days after sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which had previously been sterilized using an autoclave. Fungal isolates were grown in a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. This suspension was used as inoculum by pouring 1×10^6 cfu g^{-1} soil in a soil medium, while the uninoculated control was watered only with sterile distilled water. Each isolate was inoculated into the soil of 10 test plants and the experiment was repeated once. Disease incidence was counted by the number of diseased plants out of the 10 tested plants. The severity of the disease was calculated for each seedling using a score of 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1–30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Fungal isolates were grown in a liquid medium of PDB in a cultivation bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). Sections of the 3–4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB liquid medium and incubated for 3–4 more days at room temperature. Meanwhile, the fungal mycelium was harvested using vacuum filtration and then it was frozen. The DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA) following manufacturer's instructions. Its concentration and quality

were determined by spectrophotometry using a NanoDrop Spectrophotometry ND-1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at -20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (forward: 5′-GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio- rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 583 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using the subtree pruning regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

RESULTS AND DISCUSSION

Results

Disease symptoms and wilt incidences

149 Surveys on seedling wilt were carried out in six acacia nursery locations in
150 commercial companies, with five sites in the Air Sugihan area and one site in Lebong
151 Hitam (Table 1). The incidence of disease varied between locations from as low as
152 5.6% up to 36.9%. The early symptoms of seedling wilt started from the lower leaves
153 as they turned yellow, and then black, dried out, fell and the plant died. Some symptoms
154 began with yellowish discoloration of the leaves, which then wilted, and some had no
155 discoloration but immediately began with wilting (Fig. 1). Observations were made by
156 planting acacia on used soil medium from the commercial nursery, and the results
157 showed that 56.25% of the seedlings were attacked. In the commercial nursery, this
158 disease was found to attack acacia seedlings at an average age of more than 1 month
159 after germination and before the formation of phyllodes or false leaves.
160

161 ***Morphological characteristic***

162 Fifteen isolates similar to *F. oxysporum* were isolated from the root tissue of
163 diseased plants (Table 2) and grown on PDA and CLA media (water agar medium with
164 carnation leaf pieces) at a temperature of 27 °C. The cultures on the PDA medium
165 produced several color pigments, such as purple, pale purple and reddish-pink, with air
166 hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the
167 fastest on BF05 and FF15 isolates (11.38 mm/day). BF06 showed the slowest colony
168 growth rate (7.46 mm/day) compared to the others (Table 3). They all produce many
169 microconidia on the false heads of monophialides. Hyaline microconidia are oval,
170 elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 μm × 2.8 ± 0.5 μm to
171 11.8 ± 4.1μm × 3.0 ± 0.3 μm, and have 0 to 1 septum, but generally 0. The hyaline
172 macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 μm

173 to39.7 ± 5.9 × 3.8 ± 0.4 μm, and have 3 to 7 septa, but generally 3. The chlamydospores
174 produced singly/in pairs at the terminal/intercalary have an average diameter of 6.4 ±
175 0.6 μm to 10.0 ± 2.5 μm (Table 4). Based on the morphological characteristics of the
176 PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as
177 described by Leslie and Summerell (2006).
178
179 ***Molecular characteristics***
180 The molecular identification achieved by sequencing the *tef1* gene was
181 amplified using primers EF1 and EF2 against seven selected isolates. The sequence
182 data was determined by making a comparison with those existing in Genbank through
183 the BLAST and FUSARIUM-ID programs. Based on this, the sequences were
184 confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3−99.7%
185 with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index
186 0.9 and the composite index 0.7) grouped all *Fusarium* isolates within the *F. oxysporum*
187 species complex. Isolates were clustered within four clades (Fig. 4). The first clade
188 consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera*
189 *trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The second clade
190 consisted of single isolates from *A. mangium* (DF11) and *F. oxysporum* (C009W and
191 C010W) from *Cucumis melo*. The third consisted of DF12 and an isolate of *F.*
192 *oxysporum* from *Musa* sp. The fourth clade consisted of BF05 and EF14, and *F.*
193 *oxysporum* (CAV189) from *Musa* sp. var. Harare.
194
195 ***Pathogenicity tests***
196 *Fusaria*, isolated from diseased plant tissue, were tested for their pathogenicity in 30-
197 day-old *A. mangium* seedlings. The results showed that the symptom development

198 began with yellowing of the lower leaves or those closest to the base of the stem, these
199 then wilted, curved upward, turned brown to black, became dry, and fell from the plant.
200 This symptom progresses to the top of the plant causing it to wither and die.
201 Furthermore, infected plants show symptoms of stunted plant growth. Initial symptoms
202 appear 7–14 days after inoculation and develop, causing the infected plants to die in an
203 average range of 1 – 10 days. However, some plants experienced complete wilting
204 immediately, without any initial symptoms (Fig. 3). The control plants were healthy
205 and did not show any wilting symptoms. All the isolates were able to infect plants and
206 disease incidence and disease severity were significantly higher compared to the
207 control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease
208 severity and disease progress and their AUDPC values were significantly higher than
209 other isolates and the control (Table 4). Concerning disease incidence, the *Fusarium*
210 isolate presented a high percentage of disease incidence, ranging from 50–100%. ~~This~~
211 isolate also induced wilting ~~severity~~ ranging from 1.2 – 3.2. The highest disease
212 incidence was caused by isolate BF06 (100%), while the highest disease severity was
213 caused by isolate DE11 (3.2). Based on disease severity, the isolates were grouped into
214 three categories: high (score 2–4), moderate (score 1.2–2), and low score (0–1.2)
215 ~~virulence~~ (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06,
216 and DF11 were included in the high virulence group (2.1–3.2), while FF15, BF09,
217 BF07, AF03, and BF05 were in the moderate (1.6–2.0), and only EF14 was in the low.
218 We observed that this pathogen causes vascular disease, where the pathogen could be
219 isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the
220 pathogens were all re-isolated (100%) from infected plants and it was confirmed that
221 the morphological characters of the pathogen were the same as the inoculated *Fusarium*
222 isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *F. oxysporum* was identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous studies, *F. oxysporum* has been reported to be the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack acacia germinated seeds resulting in damping-off and the identification of this was made solely based on morphological characters (Widyastuti et al. 2013). This research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana.*

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90–100% and scoring 2.1–3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in India with diseases severity of 16.86% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85% severity (Gardner 1980), and damping-off on *A. mangium* in Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots then enter and multiply along the xylem vessel, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

247 *F. oxysporum* was determined based on morphological identification, both
248 visually and microscopically. Visually, the fungus produces pale purple to pale pink
249 pigments on a PDA medium. Microscopically, the shape of its reproductive structure is
250 the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and
251 Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-
252 causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which
253 causes acacia seedlings to wilt, is the that most similar to the *F. oxysporum* isolates
254 from *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo*
255 (Bakar and Mohd 2019), *Musa* sp., and *Musa* sp. var. Harare (Fourie et al. 2009).
256 The field observation of the disease in the commercial nursery showed that
257 about 36.9% of plants died through *Fusarium* wilt. This attack caused huge economic
258 losses because these nurseries provided seedlings for commercial gardening in South
259 Sumatra totaling 1,324,653 hectares. The high disease incidence in the field was
260 probably caused by the use of ~~previous~~ previous seedlings infested with the pathogens, which
261 led to rapid development and accumulation through the nursery cycle. This is evident
262 in the high disease incidence on soil media. It is likely that clonal dispersion occurred
263 via the nursery medium to the commercial field nursery as shown by isolates within the
264 first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al.
265 (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt
266 comes from infested soil, ~~where~~ and that the pathogen can survive a long time in the soil. The
267 pathogens can also survive in other plant debris (Postic et al. 2012; Altinok 2013)
268
269 **Conclusion**
270 This study presents the first report of *F. oxysporum* as a causal agent of *A.*
271 *mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed

through morphological and *tef1* gene sequencing and Koch's postulate. The main source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from a soil seedling medium infested with pathogens.

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020

REFERENCES

Altinok HH (2013) Fusarium Species Isolated from Common Weeds in Eggplant Fields and Symptomless Hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161: 335–340.

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f . sp . *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150: 609–621.

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4): 166–172.

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. Archives of Phytopathology and Plant Protection 43(2): 174–190.

Edel-Hermann V, Lecomte C (2019) Current Status of *Fusarium oxysporum* Formae Speciales and Races. J Phytopathol 109: 512–530.

Fourie G, Steenkamp ET, Gordon TR, Viljoen A (2009) Evolutionary relationships among the *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups. Appl Environ Microbiol 75(14): 4770–4781.

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp.

304 *koae*, f. sp. nov. J Phytopathol 70(7): 594–597.

305 Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev
306 Phytopathol 55: 23–39.

307 Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd - A Fast Growing Tree
308 for Tropical Plantation. J For Sci 29(1): 1–14.

309 Jiménez-Díaz RM, Castillo P, Jiménez-Gasco MdelM, Landa BB, Navas-Cortés JA
310 (2015) Fusarium wilt of chickpeas: Biology, ecology and management. Crop
311 Protection 73: 16–27.

312 Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused
313 by *Fusarium oxysporum*. Journal of Tropical Forest Science 16(4): 453–462.

314 Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of
315 Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3):
316 442–454.

317 Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni
318 M (2016) *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root
319 and stem rot of vanilla. Plant Pathol 65(4): 612–625.

320 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics
321 Analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870–1874.

322 Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell
323 Publishing, Oxford.

324 Luo X, Yu C (2020) First report of damping-off disease caused by *Fusarium*
325 *oxysporum* in *Pinus massoniana* in China. Journal Plant Diseases and Protection

127: 401–409.

Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium* sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. Journal of Applied Research on Medicinal and Aromatic Plants 100244.

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana : Concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Sci USA 95: 2044–2049.

Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt, focusing on bananas. Appl Soil Ecol 132: 20–33.

Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium Species Isolated from Weeds and Plant Debris in Croatia. J Phytopathol 160: 76–81.

Statistics Indonesia (2018) Timber Culture Establishment. Statistics Indonesia, Jakarta.

Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Protection 117: 77–85.

Suwandi, Akino S, Kondo N (2012) Common Spear Rot of Oil Palm in Indonesia Common Spear Rot of Oil Palm in Indonesia. Plant Dis 96(4): 537-543.

Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant Pathol 40(2): 238–247.

348 Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum*
349 fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2):
350 110–118.

351 Table 1 Disease incidence in the commercial nursery fields of *A. mangium* forestry in South

352 Sumatra

No.	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1.	Air Sugihan A	500	34	6.8
2.	Air Sugihan B	720	70	13.5
3.	Air Sugihan C	720	131	18.2
4.	Air Sugihan D	720	266	36.9
5.	Lebong Hitam	604	36	5.6
6.	Air Sugihan F	720	139	19.3

353

354 Table 2 Isolate origin used for pathogenicity test.

Location	Host	Isolate	Number of isolates
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

355

356 Table 3 Cultural characteristics of *Fusarium oxysporum* obtained from Acacia seedling wilt

357 disease.

Isolate	Colony color	Growth rate (mm/day)
AF01	Top: White to dark pink Bottom: dark pink	11.2 ± 0.6e
AF02	Top: White to pale violet Bottom: Pale violet	10.7 ± 0.6d
AF03	Top: white Bottom: pale violet	10.7 ± 0.5d
AF04	Top: white Bottom: Pale violet	11.1 ± 0.9e
BF05	Top: White to pale violet Bottom: Violet	11.4 ± 0.8e
BF06	Top: White to pale violet Bottom: Violet	7.5 ± 0.7a
BF07	Top: White to dark pink Bottom: Dark pink	7.7 ± 0.6ab
BF08	Top: White to pale violet Bottom: Pale violet	9.0 ± 0.2abc
BF09	Top: White Bottom: Violet to pale violet	8.1 ± 0.6abc
CF10	Top: White to dark pink Bottom: dark pink	9.0 ±1.5bc
DF11	Top: White to dark pink Bottom: dark pink	9.5 ± 0.4cd
DF12	Top: white to pale violet Bottom: pale violet	8.4 ± 0.5abc
DF13	Top: white to pale violet Bottom: pale violet	8.2 ± 0.4abc
EF14	Top: white Bottom: pale violet	9.3 ± 0.8cd
FF15	Top: White Bottom: Violet	11.4 ± 2.1e
LSD 0.05		2.04

358 The numbers followed by the same letter in the column are not significantly different in the LSD

359 test at 5%.

360 Table 4 Characteristics of the microscopic structure of *Fusarium oxysporum* isolated from infected plants.

Isolate	Microconidia (μm)		Macroconidia (μm)		Chlamydospore (μm)	
	Characteristics	**Size (L × W)**	**Characteristics**	**Size (L × W)**	**Characteristics**	**Size (D)**
AF01	Ellipse–allantoid, 0–1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3–4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Oval–reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3–4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Ellipse–oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3–4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Ellipse–reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3–4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Ellipse–allantoid, 0–1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3–4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Allantoid shaped, 0–2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3–4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Oval–allantoid shaped, 0–2 septa, mostly 0–1septum	7.6 ± 1.8 × 3.5 ± 0.3	3–4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Allantoid shaped, 0–2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3–4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Oval–ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3–4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Ellipse–allantoid, 0–1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3–7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Ellipse–allantoid, 0–1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3–4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Ellipse–allantoid shaped, 0–1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3–4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Ellipse–allantoid, 0–1 septum, mostly 0 septum	10. 3 ± 2.7 × 2.5 ± 0.3	3–4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7
EF14	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3–4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Oval–ellipse, 0–1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3–4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
Mean		8.1 ± 1.7 × 2.8 ± 0.3		37.5 ± 5.6 × 3.9 ± 0.4		7.7 ± 1.0

361

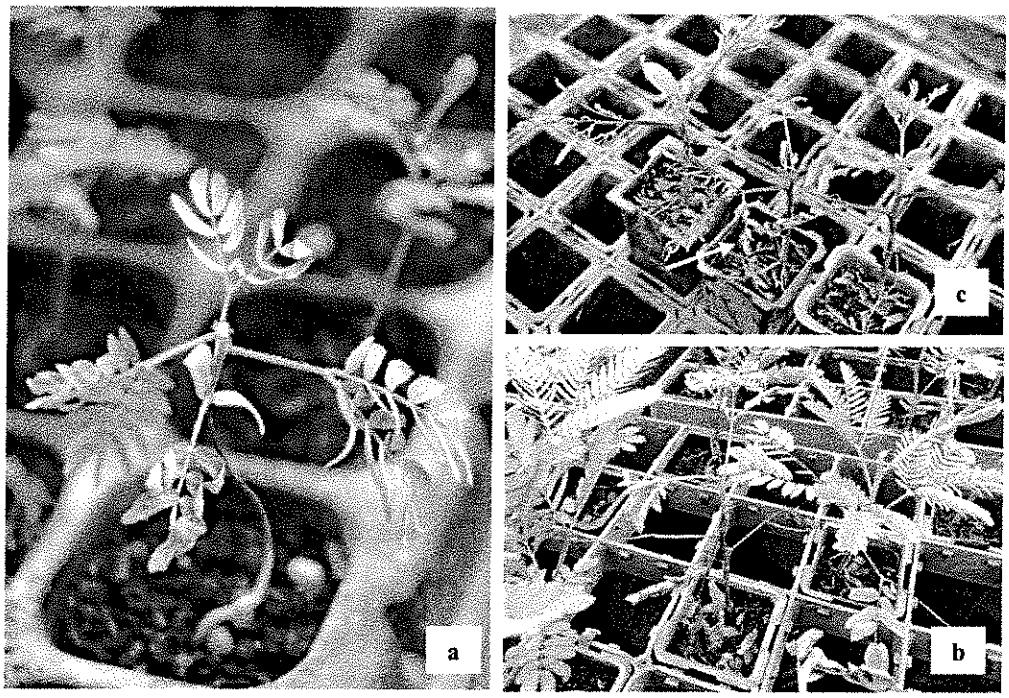
362 Table 5 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings

363 inoculated with *F. oxysporum*.

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 ab	31.0 ab
AF02	80	2.4 a	46.4 a
AF03	80	1.9 ab	32.6 ab
AF04	80	2.5 a	33.9 ab
BF05	50	2.0 ab	45.5 ab
BF06	100	3.1 a	59.5 a
BF07	60	1.9 ab	32.6 ab
BF08	70	2.1 ab	37.5 ab
BF09	70	1.6 ab	23.6 ab
CF10	90	2.7 a	37.9 a
DF11	90	3.2 a	55.7 a
DF12	80	2.8 a	49.8 a
DF13	80	2.9 a	54.7 a
EF14	60	1.2 ab	15.3 ab
FF15	50	1.6 ab	32.0 ab
Control	0	0 b	0.0 b
P-Value		0.0013	0.0007

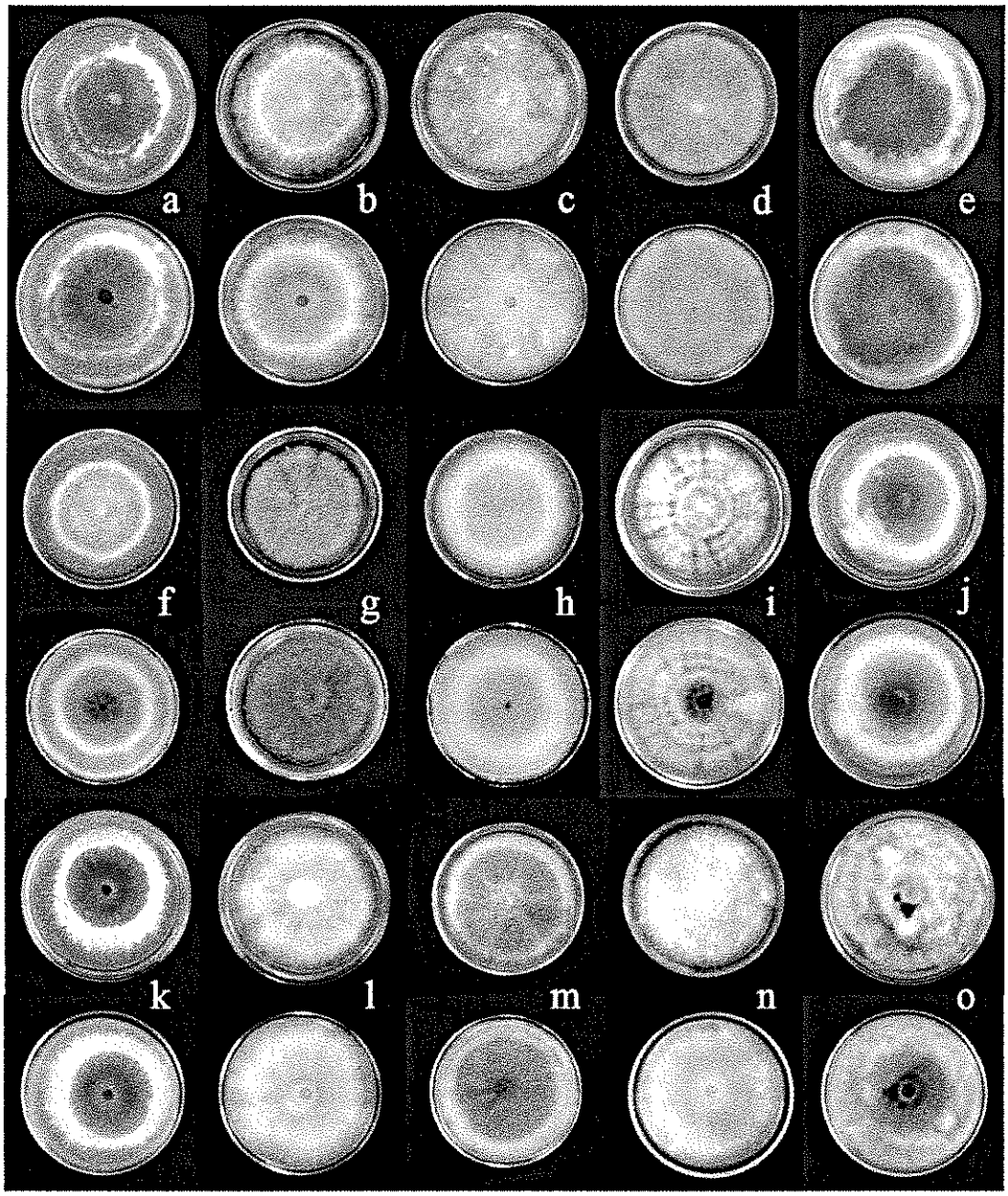
364 The numbers followed by the same letter in the column are not significantly different in the LSD

365 test at 5%.



366

367 Fig. 1 Wilt symptoms in the nursery (a) yellowing leaves (b) dry leaves (c).



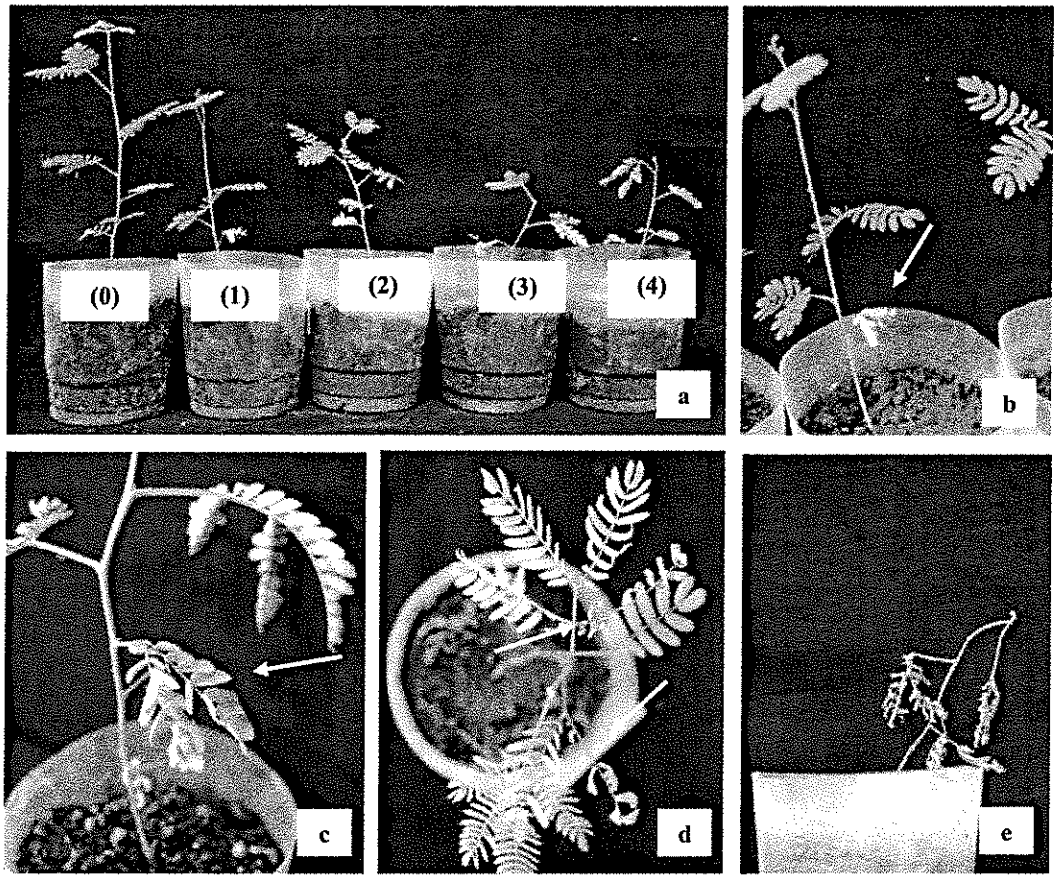
368

369 Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f),

370 BF07 (g), BF08 (h), BF09 (i), CF10 (j), DF11 (k), DF12 (l), DF13 (m), EF14 (n), and FF15 (o).

371 Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).

372

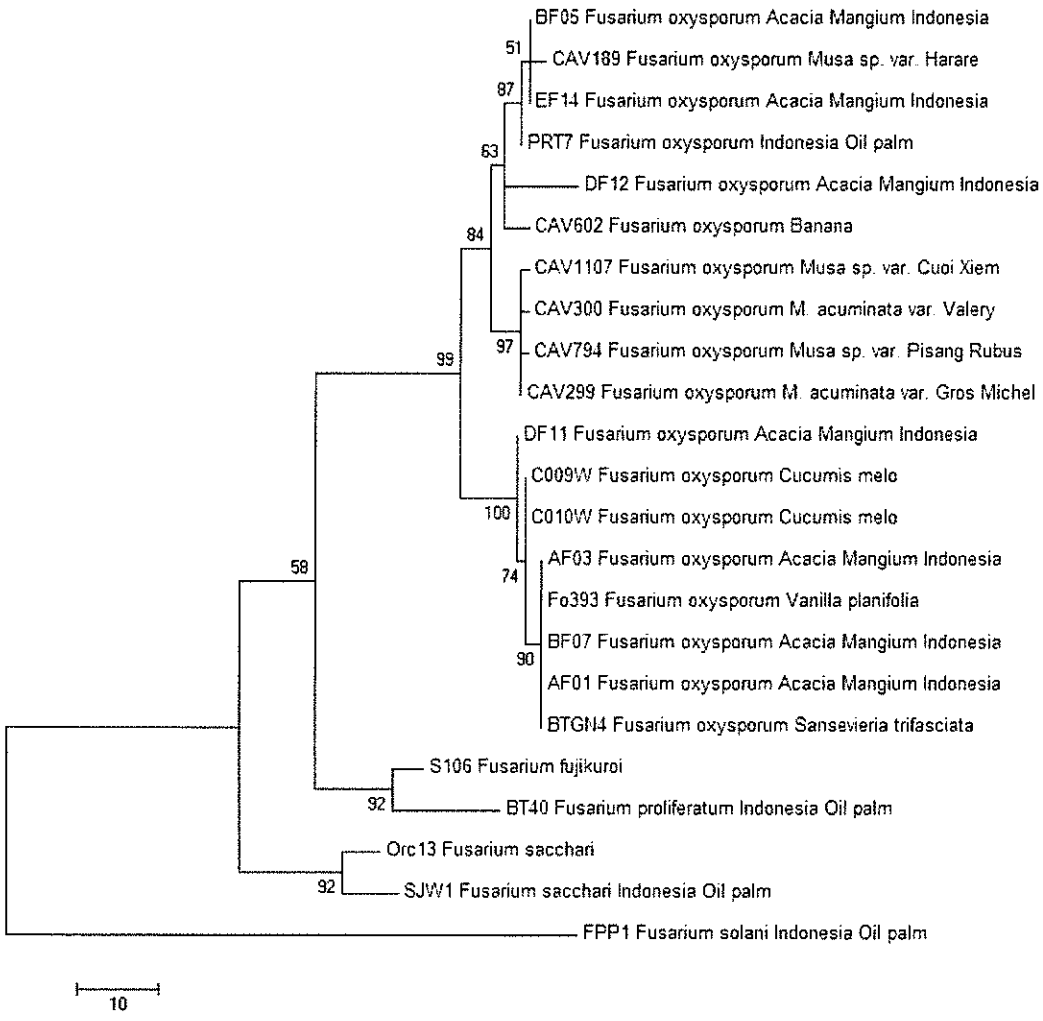


373

374 Fig. 3 Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial

375 symptoms: from lowest leaf, yellowing leaves (b). Advanced symptoms: curved leaves, dry leaves,

376 falling leaves (c,d). and dead plant (e).



377

378 Fig. 4 One out of the six most parsimonious trees showing the genetic relatedness, represented by
379 the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold), closely related
380 *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host
381 species and countries of origin are given with the representative isolates. A bootstrap test with
382 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The
383 genetic distance is indicated by the scale bar.

Journal of Forestry Research

The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease

--Manuscript Draft--

Manuscript Number:	JFR-D-21-00042
Full Title:	The Identification and Pathogenicity of Fusarium oxysporum causing Acacia Seedling Wilt Disease
Article Type:	Original Article
Section/Category:	Forest entomology and pathology
Keywords:	Acacia mangium; Fusarium oxysporum; Seedling wilt; pathogenicity
Manuscript Region of Origin:	INDONESIA
Abstract:	Fusarium oxysporum is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; consequently, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in Acacia mangium and its pathogenicity. To achieve this, 15 isolates of F. oxysporum with varying colony size and color pigment were confirmed by observing the morphological characters and elongation factor 1-α (tef1-α) gene sequences. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the Fusarium pathogen was verified as causing vascular disease. Furthermore, Koch's postulate was confirmed by re-isolating the F.oxysporum isolate. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

The Identification and Pathogenicity of *Fusarium oxysporum* causing Acacia Seedling Wilt Disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3]Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

Abstract

Fusarium oxysporum is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; consequently, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F. oxysporum* with varying colony size and color pigment were confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the *Fusarium pathogen* was verified as causing vascular disease. Furthermore, Koch's postulate was confirmed by re-isolating the *F.oxysporum* isolate. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

Keyword: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

INTRODUCTION

Acacia mangium is a green leafy plant species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable environmental conditions, this species has a high growth rate, up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies because it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia were 8.67 million hectares, with 81.30% being used for plant cultivation, including *A. mangium.* Furthermore, in 2017, this plant produced the most logs, reaching 77.55% from a total of 40,628.78 m^3 (Statistics Indonesia 2018). The problem faced in cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stage in the process of plants and, if ignored, it will have an impact caused by disease spread in the field.

F. oxysporum is a soil-borne pathogen with a very wide host range and is common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019) including forest and industrial plants (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and generative) and is able to survive on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *Acacia koae* in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F. oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F. oxysporum*, has been found to cause damping-off disease in seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced stage seedlings of *A. mangium* and the disease's pathogenicity.

MATERIAL AND METHODS

Survey and sampling

Soil and diseased plant samples were collected from five commercial company-owned acacia nursery estates with a seedling wilt problem. The diseased plants showed initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Plant samples showing wilting symptoms were collected and stored in a cool box during the process. To determine soil infectivity, acacia seeds were sown using a seedbed, and to accelerate germination, they were soaked in hot water (± 95 °C) and left to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate the radicula germination. The germinated seeds were planted on infested field nursery medium. The infected seedlings from the field and the infected soil nursery medium were taken and the pathogen was isolated from the plant tissue.

Commented [USER1]: How to sample?

Commented [USER2]: Where is the exact location? With GPS?

Commented [USER3]: With roots? Or part of plants?

Fungal isolation

Fungi were isolated from the roots of plants showing the symptoms of seedling wilt, both from the field and the soil infected with pathogens. Then, the root samples were washed under running water, and the surface was sterilized by dipping them in a solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et al. 2012). Then, they were planted in a Petri plate containing agar water with 2%(w/v) agar and 0.1% Streptomycin-sulfate and incubated for 2 × 24 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on the Leslie and Summerell (2006) method. The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed based on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia, macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

A pathogenicity test was carried out on *A. mangium* seedlings 30 days after sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which had previously been sterilized using an autoclave. Fungal isolates were grown in a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a soil medium, while the uninoculated control was watered only with sterile distilled water. Each isolate was inoculated on 10 test plants and the experiment was repeated once. Disease incidence was counted by the number of diseased plants out of the 10 tested plants. The severity of the disease was calculated using a score of 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1–30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3 4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB liquid medium and incubated for 3–4 days at room temperature. Meanwhile, the fungal mycelium was harvested using vacuum filtration and then it was frozen. The DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA) following manufacturer's instructions. Its concentration and quality

were determined by spectrophotometry using a NanoDrop Spectrophotometry ND-1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at -20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (forward: 5′ GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio-rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 583 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using the subtree pruning regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

RESULTS AND DISCUSSION

Results

Disease symptoms and wilt incidences

149 Surveys on seedling wilt were carried out in six acacia nursery locations in
150 commercial companies, with five sites in the Air Sugihan area and one site in Lebong
151 Hitam (Table 1). The incidence of disease varied between locations from as low as
152 5.6% up to 36.9%. The early symptoms of seedling wilt started from the lower leaves
153 as they turned yellow, and then black, dried out, fell and the plant died. Some symptoms
154 began with yellowish discoloration of the leaves, which then wilted, and some had no
155 discoloration but immediately began with wilting (Fig. 1). Observations were made by
156 planting acacia on used soil medium from the commercial nursery, and the results
157 showed that 56.25% of the seedlings were attacked. In the commercial nursery, this
158 disease was found to attack acacia seedlings at an average age of more than 1 month
159 159 after germination and before the formation of phyllodes or false leaves.
160 160

161 Morphological characteristic

162 Fifteen isolates similar to *F. oxysporum* were isolated from the root tissue of
163 diseased plants (Table 2) and grown on PDA and CLA media (water agar medium with
164 carnation leaf pieces) at a temperature of 27 °C. The cultures on the PDA medium
165 produced several color pigments, such as purple, pale purple and reddish-pink, with air
166 hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the
167 fastest on BF05 and FF15 isolates (11.38 mm/day). BF06 showed the slowest colony
168 growth rate (7.46 mm/day) compared to the others (Table 3). They all produce many
169 microconidia on the false heads of monophialides. Hyaline microconidia are oval,
170 elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm to
171 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have 0 to 1 septum, but generally 0. The hyaline
172 macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm

Commented [USER4]: How it can be? Commonly the microconidia of Fusarium is not reniform

to39.7 ± 5.9 × 3.8 ± 0.4 µm, and have 3 to 7 septa, but generally 3. The chlamydospores produced singly/in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 4). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

The molecular identification achieved by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by making a comparison with those existing in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3−99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.7) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 4). The first clade consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The second clade consisted of single isolates from *A. mangium* (DF11) and *F. oxysporum* (C009W and C010W) from *Cucumis melo*. The third consisted of DF12 and an isolate of *F. oxysporum* from *Musa* sp. The fourth clade consisted of BF05 and EF14, and *F. oxysporum* (CAV189) from *Musa* sp. var. Harare.

Pathogenicity tests

Fusaria, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A. mangium* seedlings. The results showed that the symptom development

198 began with yellowing of the lower leaves or those closest to the base of the stem, these
199 then wilted, curved upward, turned brown to black, became dry, and fell from the plant.
200 This symptom progresses to the top of the plant causing it to wither and die.
201 Furthermore, infected plants show symptoms of stunted plant growth. Initial symptoms
202 appear 7−14 days after inoculation and develop, causing the infected plants to die in an
203 average range of 1 − 10 days. However, some plants experienced complete wilting
204 immediately, without any initial symptoms (Fig. 3). The control plants were healthy
205 and did not show any wilting symptoms. All the isolates were able to infect plants and
206 disease incidence and disease severity were significantly higher compared to the
207 control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease
208 severity and disease progress and their AUDPC values were significantly higher than
209 other isolates and the control (Table 4). Concerning disease incidence, the *Fusarium*
210 isolate presented a high percentage of disease incidence, ranging from 50−100%. This
211 isolate also induced wilting severity ranging from 1.2−3.2. The highest disease
212 incidence was caused by isolate BF06 (100%), while the highest disease severity was
213 caused by isolate DF11 (3.2). Based on disease severity, the isolates were grouped into
214 three categories: high (score 2 − 4), moderate (score 1.2 − 2), and low score (0 − 1.2)
215 virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06,
216 and DF11 were included in the high virulence group (2.1 − 3.2), while FF15, BF09,
217 BF07, AF03, and BF05 were in the moderate (1.6−2.0), and only EF14 was in the low.
218 We observed that this pathogen causes vascular disease, where the pathogen could be
219 isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the
220 pathogens were all re-isolated (100%) from infected plants and it was confirmed that
221 the morphological characters of the pathogen were the same as the inoculated *Fusarium*
222 isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *F. oxysporum* was identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous studies, *F. oxysporum* has been reported to be the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack acacia germinated seeds resulting in damping-off and the identification of this was made solely based on morphological characters (Widyastuti et al. 2013). This research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana.*

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in India with diseases severity of 16.86% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85% severity (Gardner 1980), and damping-off on *A. mangium* in Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots then enter and multiply along the xylem vessel, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

F. oxysporum was determined based on morphological identification, both visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the that most similar to the *F. oxysporum* isolates from *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar and Mohd 2019), *Musa* sp., and *Musa* sp. var. Harare (Fourie et al. 2009).

The field observation of the disease in the commercial nursery showed that about 36.9% of plants died through *Fusarium* wilt. This attack caused huge economic losses because these nurseries provided seedlings for commercial gardening in South Sumatra totaling 1,324,653 hectares. The high disease incidence in the field was probably caused by the use of previous seedlings infested with the pathogens, which led to rapid development and accumulation through the nursery cycle. This is evident in the high disease incidence on soil media. It is likely that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt comes from infested soil, where the pathogen can survive a long time in the soil. The pathogens can also survive in other plant debris (Postic et al. 2012; Altinok 2013)

Conclusion

This study presents the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed

through morphological and *tef1* gene sequencing and Koch's postulate. The main source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from a soil seedling medium infested with pathogens.

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020

REFERENCES

Altinok HH (2013) Fusarium Species Isolated from Common Weeds in Eggplant Fields and Symptomless Hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161: 335–340.

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f . sp . *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150: 609–621.

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4): 166–172.

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. Archives of Phytopathology and Plant Protection 43(2): 174–190.

Edel-Hermann V, Lecomte C (2019) Current Status of *Fusarium oxysporum* Formae Speciales and Races. J Phytopathol 109: 512–530.

Fourie G, Steenkamp ET, Gordon TR, Viljoen A (2009) Evolutionary relationships among the *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups. Appl Environ Microbiol 75(14): 4770–4781.

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp.

304 *koae*, f. sp. nov. J Phytopathol 70(7): 594–597.

305 Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev
306 Phytopathol 55: 23–39.

307 Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd - A Fast Growing Tree
308 for Tropical Plantation. J For Sci 29(1): 1–14.

309 Jiménez-Díaz RM, Castillo P, Jiménez-Gasco MdelM, Landa BB, Navas-Cortés JA
310 (2015) Fusarium wilt of chickpeas: Biology, ecology and management. Crop
311 Protection 73: 16–27.

312 Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused
313 by *Fusarium oxysporum*. Journal of Tropical Forest Science 16(4): 453–462.

314 Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of
315 Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3):
316 442–454.

317 Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni
318 M (2016) *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root
319 and stem rot of vanilla. Plant Pathol 65(4): 612–625.

320 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics
321 Analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870–1874.

322 Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell
323 Publishing, Oxford.

324 Luo X, Yu C (2020) First report of damping‐off disease caused by *Fusarium*
325 *oxysporum* in *Pinus massoniana* in China. Journal Plant Diseases and Protection

326 127: 401–409.

327 Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium*
328 sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management
329 strategies. Journal of Applied Research on Medicinal and Aromatic Plants 100244.

330 Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University
331 Press, New York.

332 O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins
333 of the fungus causing Panama disease of banana: Concordant evidence from
334 nuclear and mitochondrial gene genealogies. Proc Natl Sci USA 95: 2044–2049.

335 Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt, focusing
336 on bananas. Appl Soil Ecol 132: 20–33.

337 Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of
338 Fusarium Species Isolated from Weeds and Plant Debris in Croatia. J Phytopathol
339 160: 76–81.

340 Statistics Indonesia (2018) Timber Culture Establishment. Statistics Indonesia, Jakarta.

341 Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as
342 a causal agent of mung bean wilt in China. Crop Protection 117: 77–85.

343 Suwandi, Akino S, Kondo N (2012) Common Spear Rot of Oil Palm in Indonesia
344 Common Spear Rot of Oil Palm in Indonesia. Plant Dis 96(4): 537-543.

345 Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-
346 Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper
347 in Mexico. Can J Plant Pathol 40(2): 238–247.

348 Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum*
349 fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2):
350 110–118.

Table 1 Disease incidence in the commercial nursery fields of *A. mangium* forestry in South Sumatra

No.	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1.	Air Sugihan A	500	34	6.8
2.	Air Sugihan B	720	70	13.5
3.	Air Sugihan C	720	131	18.2
4.	Air Sugihan D	720	266	36.9
5.	Lebong Hitam	604	36	5.6
6.	Air Sugihan F	720	139	19.3

Table 2 Isolate origin used for pathogenicity test.

Location	Host	Isolate	Number of isolates
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

356 Table 3 Cultural characteristics of *Fusarium oxysporum* obtained from Acacia seedling wilt

357 disease.

Isolate	Colony color	Growth rate (mm/day)
AF01	Top: White to dark pink Bottom: dark pink	11.2 ± 0.6e
AF02	Top: White to pale violet Bottom: Pale violet	10.7 ± 0.6d
AF03	Top: white Bottom: pale violet	10.7 ± 0.5d
AF04	Top: white Bottom: Pale violet	11.1 ± 0.9e
BF05	Top: White to pale violet Bottom: Violet	11.4 ± 0.8e
BF06	Top: White to pale violet Bottom: Violet	7.5 ± 0.7a
BF07	Top: White to dark pink Bottom: Dark pink	7.7 ± 0.6ab
BF08	Top: White to pale violet Bottom: Pale violet	9.0 ± 0.2abc
BF09	Top: White Bottom: Violet to pale violet	8.1 ± 0.6abc
CF10	Top: White to dark pink Bottom: dark pink	9.0 ±1.5bc
DF11	Top: White to dark pink Bottom: dark pink	9.5 ± 0.4cd
DF12	Top: white to pale violet Bottom: pale violet	8.4 ± 0.5abc
DF13	Top: white to pale violet Bottom: pale violet	8.2 ± 0.4abc
EF14	Top: white Bottom: pale violet	9.3 ± 0.8cd
FF15	Top: White Bottom: Violet	11.4 ± 2.1e
LSD 0.05		2.04

358 The numbers followed by the same letter in the column are not significantly different in the LSD

359 test at 5%.

360 Table 4 Characteristics of the microscopic structure of *Fusarium oxysporum* isolated from infected plants.

Isolate	Microconidia (μm)		Macroconidia (μm)		Chlamydospore (μm)	
	Characteristics	Size (L × W)	Characteristics	Size (L × W)	Characteristics	Size (D)
AF01	Ellipse–allantoid, 0–1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3–4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Oval–reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3–4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Ellipse–oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3–4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Ellipse–reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3–4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Ellipse–allantoid, 0–1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3–4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Allantoid shaped, 0–2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3–4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Oval–allantoid shaped, 0–2 septa, mostly 0–1septum	7.6 ± 1.8 × 3.5 ± 0.3	3–4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Allantoid shaped, 0–2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3–4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Oval–ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3–4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Ellipse–allantoid, 0–1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3–7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Ellipse–allantoid, 0–1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3–4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Ellipse–allantoid shaped, 0–1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3–4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Ellipse–allantoid, 0–1 septum, mostly 0 septum	10. 3 ± 2.7 × 2.5 ± 0.3	3–4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7
EF14	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3–4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Oval–ellipse, 0–1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3–4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
					Mean	8.1 ± 1.7 × 2.8

361

±

0

3

37.5 ± 5.6 ×
3.9 ± 0.4

7.7 ± 1.0

362 Table 5 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings

363 inoculated with *F. oxysporum*.

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 ab	31.0 ab
AF02	80	2.4 a	46.4 a
AF03	80	1.9 ab	32.6 ab
AF04	80	2.5 a	33.9 ab
BF05	50	2.0 ab	45.5 ab
BF06	100	3.1 a	59.5 a
BF07	60	1.9 ab	32.6 ab
BF08	70	2.1 ab	37.5 ab
BF09	70	1.6 ab	23.6 ab
CF10	90	2.7 a	37.9 a
DF11	90	3.2 a	55.7 a
DF12	80	2.8 a	49.8 a
DF13	80	2.9 a	54.7 a
EF14	60	1.2 ab	15.3 ab
FF15	50	1.6 ab	32.0 ab
Control	0	0 b	0.0 b
P-Value		0.0013	0.0007

364 The numbers followed by the same letter in the column are not significantly different in the LSD

365 test at 5%.

Figure

Fig. 1 Wilt symptoms in the nursery (a) yellowing leaves (b) dry leaves (c).

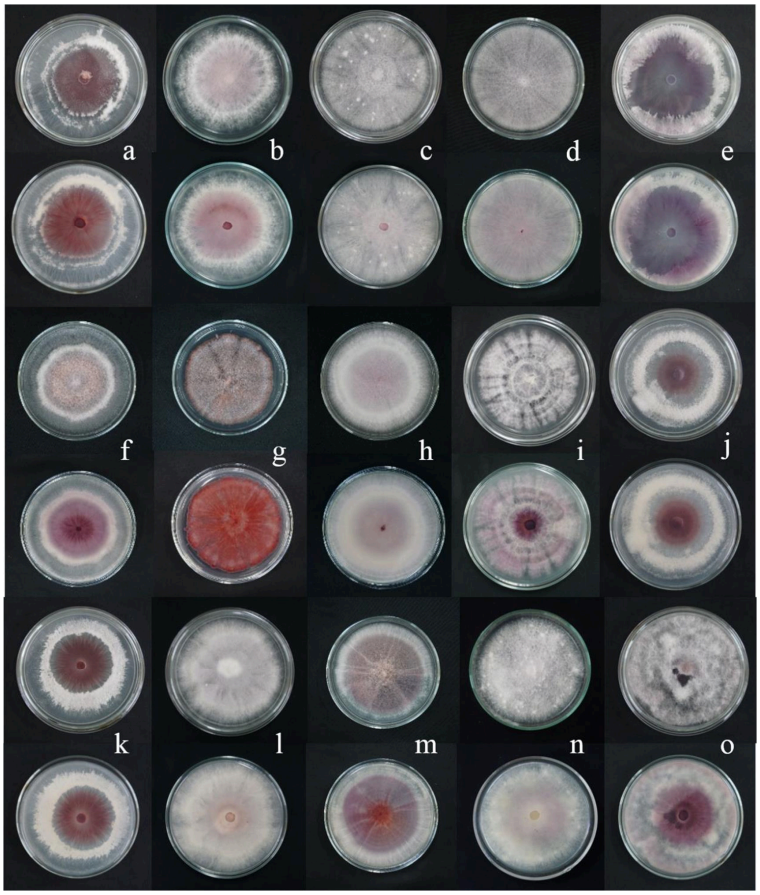


Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f), BF07 (g), BF08 (h), BF09 (i), CF10 (j), DF11 (k), DF12 (l), DF13 (m), EF14 (n), and FF15 (o). Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).

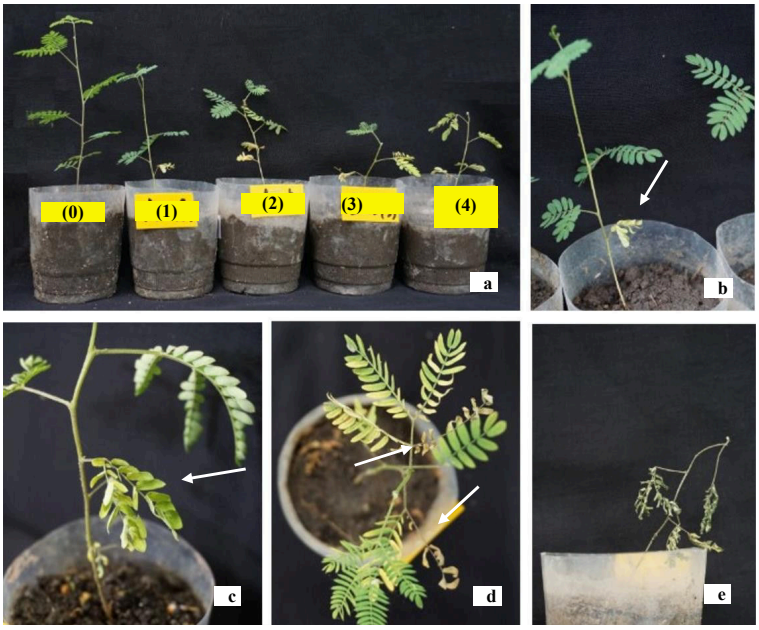
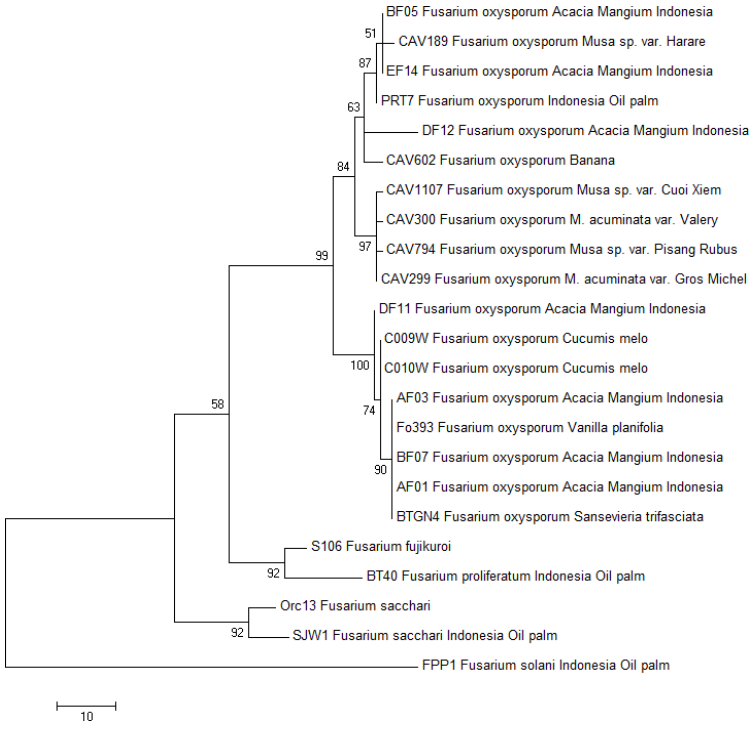


Fig. 3 Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial symptoms: from lowest leaf, yellowing leaves (b). Advanced symptoms: curved leaves, dry leaves, falling leaves (c,d). and dead plant (e).



377 377

378 Fig. 4 One out of the six most parsimonious trees showing the genetic relatedness, represented by
379 the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold), closely related
380 *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host
381 species and countries of origin are given with the representative isolates. A bootstrap test with
382 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The
383 genetic distance is indicated by the scale bar.

3. Bukti konfirmasi submit revisi, respon kepada reviewer, dan artikel yang diresubmit (05 April 2021)

The Identification and Pathogenicity of *Fusarium oxysporum* causing Acacia Seedling Wilt Disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3]Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

Abstract

~~*Fusarium oxysporum* is an important pathogen in nurseries of commercial acacia in South Sumatra, causing plant mortality of 36.94%.~~ Wilt disease with unknown etiology causes mass mortality in commercial *Acacia mangium* nursery of South Sumatra. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; subsequently~~consequently~~, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in *A.~~cacia~~ mangium* and to assess its pathogenicity. Total~~To achieve this,~~ 15 isolates of ~~-~~*F. oxysporum* with varying colony sizes and color pigments were recovered from symptomatic *A. mangium* seedlings. ~~confirmed by observing the morphological characters and elongation factor 1-α (tef1-α) gene sequences.~~ The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the *Fusarium* pathogen was verified as causing vascular disease. Furthermore, Koch's postulate were~~was~~ confirmed by re-

Formatted: Font: Not Italic

isolating the *F. -oxysporum* isolates. Pathogen was confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences as *F. oxysporum*. The primary source of pathogenic inoculums in commercial nurseries at South Sumatra comes from soil seedling media infested with pathogens.

Keyword: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

INTRODUCTION

Acacia mangium is a green leafy plant species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable environmental conditions, this species can growhas a high growth rate, up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies because it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia were 8.67 million hectares, with 81.30% being used for plant cultivation, including *A. mangium*. Furthermore, in 2017, this plant produced the most logs, reaching 77.55% from a total of 40,628.78 m^3 (Statistics Indonesia 2018). The main problem faced in cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stage of plant growthin the process of plants and, if ignored, it can spread widely in a plantation.will have an impact caused by disease spread in the field.

F. oxysporum is a soil-borne pathogen with a very wide host range and is common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019) including forest and industrial plantationsplants (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and regenerative) and is able to survive on plant debris for a long period (Postic et al. 2012; Meena and Roy

2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *Acacia koa~~e~~* in Hawaii caused by *F. oxysporum* f. sp. *koae*, f. sp. nov. (Gardner 1980; Dobbs et al. ~~,~~ 2020; ~~Gardner, 1980~~)~~(Gardner 1980)~~. Furthermore, *F. oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium* in Papua (Indonesia), *F. oxysporum~~,~~* has been found to cause damping-off disease in seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced stage seedlings of *A. mangium* and the disease's pathogenicity.

Field Code Changed

MATERIAL AND METHODS

Survey and sampling

Soil and diseased plant samples were collected from six~~five~~ commercial company-owned acacia nursery estates with a seedling wilt problem. The diseased plants showed initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Plant samples showing wilting symptoms were collected and stored in a cool box during the process. To determine soil infectivity, acacia seeds were sown using a seedbed, and to accelerate germination, they were soaked in hot water (± 95 ˚C) and left to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15

min, and rinsed three times with sterile distilled water. They were then incubated for ~~2 × 24~~48 h to accelerate the radicul development~~radicula germination~~. The germinated seeds were planted on infested field nursery medium. The infected seedlings from the field and the infested~~infected~~ soil nursery medium were sampled~~taken~~ and the pathogen was also isolated from the plant tissue.

Fungal isolation

Fungi were isolated from the roots of plants growing in the field that showed wilt symptoms and also from~~showing the symptoms of seedling wilt, both from the field and the~~ soil infested~~infected~~ with pathogens. Then, the root samples were washed under running water, and the surface was sterilized by dipping them in a solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et al. 2012). Then, they were planted in a Petri plate containing agar water with 2% (w/v) agar and 0.1% Streptomycin-sulfate and incubated for ~~2 × 24~~48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on the Leslie and Summerell (2006) method. The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed based on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX

23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia, macroconidia~~,~~ and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

A pathogenicity test was carried out on *A. mangium* seedlings 30 days after sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which had previously been sterilized using an autoclave. Fungal isolates were grown in a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. This~~Its~~ suspension was used as inoculum~~inoculated~~ by pouring 1×10^6 cfu g^{-1} soil in a soil medium, while the uninoculated control was watered only with sterile distilled water. Each isolate was inoculated into the soil of~~on~~ 10 test plants and the experiment was repeated once. Disease incidence was counted by the number of diseased plants out of the 10 tested plants. The severity of the disease was calculated for each seedling using a score of 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1−30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Seven representatives ~~F~~fungal isolates with distinct morphological characteristics were selected and grown in a liquid medium of PDB in~~on~~ a cultivation bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water) section of~~. The~~ 3–4-day old cultures~~isolates~~ on the PDA medium measuring 5 × 5 mm were placed in a PDB liquid medium and incubated for 3 – 4 more days at room temperature. Meanwhile, the fungal mycelium was harvested using vacuum filtration and then it was frozen. The DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, California, USA) following manufacturer's instructions. Its concentration and quality were determined by spectrophotometry using a NanoDrop Spectrophotometry ND - 1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at -20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (reverse~~forward~~: 5′ -GGAAGTACCAGTGATCATGTT -3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio-rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *A.~~cacia~~ mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP)

Formatted: Indent: First line: 0.5"

analyses were performed. There was a total of 57783 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using the subtree pruning regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

RESULTS AND DISCUSSION

Results

Disease symptoms and wilt incidences

Surveys on seedling wilt were carried out in six acacia nursery locations in commercial companies, with five sites in the Air Sugihan area and one site in Lebong Hitam (Table 1). The incidence of disease varied between locations from as low as 6.05.6% up to 36.9%. The early symptoms of seedling wilt started from the lower leaves as they turned yellow, and then black, dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves, which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1). Observations were made by planting acacia on used soil medium from the commercial nursery, and the results showed that 56.25% of the seedlings were attacked. In the commercial nursery, this disease was found to attack acacia seedlings at an average age of more than 1 month after germination and before the formation of phyllodes or false leaves.

Morphological characteristic

Fifteen isolates similar to *F. oxysporum* were isolated from the root tissue of diseased plants (Table 2) and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at a temperature of 27 °C. The cultures on the PDA medium

produced several color pigments, such as purple, pale purple and reddish-pink, with air hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.38 mm/day). BF06 showed the slowest colony growth rate (7.46 mm/day) compared to the others (Fig. 3~~Table 3~~). They all produce many microconidia on the false heads of monophialides. Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm to 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have 0 to 1 septum, but generally 0. The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm to39.7 ± 5.9 × 3.8 ± 0.4 µm, and have 3 to 7 septa, but generally 3. The chlamydospores produced singly/in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 3~~4~~). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

The molecular identification achieved by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by making a comparison with those existing in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3−99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.8~~7~~) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 5~~4~~). The first clade consisted of BF05 and EF14, and *F. elaeidis* (MH484961.1) from *Elaeis* sp. ~~The first clade consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum*~~

~~from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*).~~ The second clade consisted of single isolates from *A. mangium* (DF11) ~~and~~ *F. oxysporum* (C009W and C010W) from *Cucumis melo*, and *F. triseptatum* (MH484964.1) from *Ipomoea batatas*. ~~The third consisted of DF12 and an isolate of *F. oxysporum* from *Musa* sp. The fourth clade consisted of BF05 and EF14, and *F. oxysporum* (CAV189) from *Musa* sp. var. Harare.~~ The third ~~first~~ clade consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The fourth consisted of DF12 and an isolate of *F. oxysporum* from *Musa* sp. *Tef1* sequences of isolates within third and fourth clade were separated from all 15 cryptic taxa of *F. oxysporum* species complex as described by ~~(~~Lombard et al.~~, Lamprecht, & Crous,~~ (2019).

Pathogenicity tests

Fusaria, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A. mangium* seedlings. The results showed that the symptom development began with yellowing of the lower leaves or those closest to the base of the stem, these then wilted, curved upward, turned brown to black, became dry, and fell from the plant. This symptom progresses to the top of the plant causing it to wither and die. Furthermore, infected plants show symptoms of stunted plant growth. ~~Initial symptoms appear 7–14 days after inoculation and develop, causing the infected plants to die in an average range of 1–10 days~~ The first symptoms appeared 7 to 14 days after inoculation and were followed by total plant collapse within 1 to 10 days. However, some plants experienced complete wilting immediately, without any initial symptoms (Fig. 3). The control plants were healthy and did not show any wilting symptoms. All the isolates were able to infect plants and disease incidence and disease severity were significantly

higher compared to the control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease severity and disease progress and their AUDPC values were significantly higher than other isolates and the control (Table 4). Concerning disease incidence, the *Fusarium* isolates presented a high variation in percentage of disease incidence, ranging from 50 − 100%. These~~This~~ isolates also induced wilting severities~~severity~~ ranging from 1.2−3.2. The highest disease incidence was caused by isolate BF06 (100%), while the highest disease severity was caused by isolate DF11 (3.2). Based on disease severity, the isolates were grouped into three categories of virulence: high (score 2−4), moderate (score 1.2−2), and low score (0−1.2) virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11 were included in the high virulence group (2.1−3.2), while FF15, BF09, BF07, AF03, and BF05 were in the moderate (1.6−2.0), and only EF14 was in the low. We observed that this pathogen causes vascular disease, where the pathogen could be isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the pathogens were all re-isolated (100%) from infected plants and it was confirmed that the morphological characters of the pathogen were the same as the inoculated *Fusarium* isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *F. oxysporum* was identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous studies, *F. oxysporum* has been reported to be the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack acacia germinated seeds resulting in damping-off and the identification of this was made solely based on morphological

characters (Widyastuti et al. 2013). This research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana*.

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in India with diseases severity of 16.86% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85% severity (Gardner 1980), and damping-off on *A. mangium* in Papua, Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots then enter and multiply along the xylem vessel, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

F. oxysporum was determined based on morphological identification, both visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F. oxysporum*, generally exhibiting a short monophialide (Leslie and Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the that most similar to the *F. oxysporum* species complex ~~isolates~~ from *Elaeis* sp. (*F. elaeidis*) (Lombard et al.~~,~~ 2019), *S. trifasciata* (Kee

et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar and Mohd 2019)~~,~~ ~~*Musa* sp., and *Musa* sp. var. Harare (Fourie et al. 2009).~~

The field observation of the disease in the commercial nursery showed that about 36.9% of plants died through *Fusarium* wilt. This attack caused huge economic losses because these nurseries provided seedlings for commercial gardening in South Sumatra totaling 1,324,653 hectares. The high disease incidence in the field was probably caused by the previous use of ~~previous~~ seedlings infested with the pathogens, which led to rapid development and accumulation through the nursery cycle. This is evident in the high disease incidence on soil media. It is likely that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt comes from infested soil and that~~, where the~~ pathogen can survive a long time in the soil. The pathogens can also survive in other plant debris (Postic et al. 2012; Altinok 2013)

Conclusion

This study presents the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed through morphological and *tef1* gene sequencing and Koch's postulate. The main source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from a soil seedling medium infested with pathogens.

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020

REFERENCES

Altinok, H. H. (2013). Fusarium species isolated from common weeds in eggplant fields and symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. Journal of Phytopathology, 161; 335–340. https://doi.org/10.1111/jph.12074

Altinok, H. H., Can, C., & Altinok, M. A. (2018). Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. European Journal of Plant Pathology, 150; 609–621.

Bakar, M. A., & Mohd, M. (2019). Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona, L. G., Grajales, A., Cardenas, M. E., Sierra, R., Lozano, G., Garavito, M. F., … Restrepo, S. (2011). Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Revista Iberoamericana de Micología, 28(4); 166–172.

Dobbs, J. T., Kim, M. S., Dudley, N. S., Klopfenstein, N. B., Yeh, A., Hauff, R. D., … Stewart, J. E. (2020). Whole genome analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) and the development of molecular tools for early detection and monitoring. BMC Genomics, 21; 1–15.

Dubey, S. C., Singh, S. R., & Singh, B. (2010). Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f . sp . *ciceris*. Archives of Phytopathology and Plant Protection, 43(2); 174–190. https://doi.org/10.1080/03235400802021108

Edel-Hermann, V., & Lecomte, C. (2019). Current status of *Fusarium oxysporum* f. sp. and races. J Phytopathology, 109; 512–530. https://doi.org/10.1094/PHYTO-08-18-0320-RVW

Gardner, D. E. (1980). Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp.

koae, f. sp. nov. J Phytopathology, 70(7):, 594–597.

Gordon, T. R. (2017). *Fusarium oxysporum* and the Fusarium Wilt Syndrome. The Annual Review of Phytopathology, 55:, 23–39.

Hedge, M., Palanisamy, K., & Yi, J. S. (2013). *Acacia mangium* Willd . — A Fast Growing Tree for Tropical Plantations. Journal of Forest Science, 29(1):, 1–14.

Jiménez-Díaz, R. M., Castillo, P., Jiménez-Gasco, M. del M., Landa, B. B., & Navas-Cortés, J. A. (2015). Fusarium wilt of chickpeas: Biology, ecology and management. Crop Protection, 73:, 16–27. https://doi.org/10.1016/j.cropro.2015.02.023

Kapoor, S., Harsh, N. S. K., & Sharma, S. K. (2004). A new wilt disease of *Aacacia nilotica* caused by *Ffusarium oxysporum*.pdf. Journal of Tropical Forest Science, 16(4):, 453–462.

Kee, Y. J., Zakaria, L., & Mohd, M. H. (2020). Morphology, phylogeny and pathogenicity of Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant Pathology, 69(3):, 442–454. https://doi.org/10.1111/ppa.13138

Koyyappurath, S., Atuahiva, T., Le Guen, R., Batina, H., Le Squin, S., Gautheron, N., … Grisoni, M. (2016). *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla. Plant Pathology, 65(4):, 612–625. https://doi.org/10.1111/ppa.12445

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7):, 1870–1874. https://doi.org/10.2166/nh.2003.0008

Leslie, J. F., & Summerell, B. A. (2006). The Fusarium Laboratory Manual. Oxford: Blackwell Publishing, Oxford.

Lombard, L., Lamprecht, S. C., & Crous, P. W. (2019). Epitypification of *Fusarium*

Formatted: Font: Not Italic

oxysporum – clearing the taxonomic chaos~~– Epitypification of Fusarium oxysporum – clearing the taxonomic chaos.~~ Persoonia 43: 1-15.~~(January 2018).~~ ~~https://doi.org/10.3767/persoonia.2019.43.01~~

Luo~~,~~ X.~~,~~ ~~&~~ Yu~~,~~ C.~~(2020).~~ First report of damping‐off disease caused by *Fusarium oxysporum* in *Pinus massoniana* in China. Journal of Plant Diseases and Protection~~,~~ 127~~:,~~ 401–409. ~~https://doi.org/10.1007/s41348-020-00303-3~~

Meena~~,~~ R.~~–~~P.~~,~~ ~~&~~ Roy~~,~~ S.~~(2020).~~ Morphological and molecular characterization of *Fusarium* sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. Journal of Applied Research on Medicinal and Aromatic Plants~~, (July 2019), 100244. https://doi.org/10.1016/j.jarmap.2020.100244~~ 100244.

Nei~~,~~ M.~~,~~ ~~&~~ Kumar~~,~~ S.~~(2000).~~ Molecular Evolution and Phylogenetics. ~~New York:~~ Oxford University Press, New York.~~.~~

O'Donnell~~,~~ K.~~,~~ Kistler~~,~~ H.~~–~~C.~~,~~ Cigernik~~,~~ E.~~,~~ ~~&~~ Ploetz~~,~~ R.~~–~~C.~~(1998).~~ Multiple evolutionary origins of the fungus causing Panama disease of banana : Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Sci. USA~~,~~ 95~~:,~~ 2044–2049.

Orr~~,~~ R.~~,~~ ~~&~~ Nelson~~,~~ P.~~–~~N.~~(2018).~~ Impacts of soil abiotic attributes on Fusarium wilt , focusing on bananas. Appl~~ied~~ Soil Ecol~~ogy,~~ 132~~:(June),~~ 20–33. ~~https://doi.org/10.1016/j.apsoil.2018.06.019~~

Postic~~,~~ J.~~,~~ Cosic~~,~~ J.~~,~~ Vrandecic~~,~~ K.~~,~~ Jurkovic~~,~~ D.~~,~~ Saleh~~,~~ A.~~–~~A.~~,~~ ~~&~~ Leslie~~,~~ J.~~–~~F.~~(2012).~~ D~~d~~iversity of F~~f~~usarium species isolated from weeds and plant debris in Croatia. J~~ournal of~~ Phytopathol~~ogy,~~ 160~~:,~~ 76–81. ~~https://doi.org/10.1111/j.1439-0434.2011.01863.x~~

Statistics Indonesia. (2018). Timber Culture Establishment. ~~Jakarta:~~ BPS-Statistics

Indonesia, Jakarta.

Sun, F., Sun, S., Zhu, L., Duan, C., & Zhu, Z. (2019). Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Protection, 117(April 2018),: 77–85.

Suwandi, Akino, S., & Kondo, N. (2012). Ceommon spear rot of oil palm in Indonesia Common Spear Rot of Oil Palm in Indonesia. Plant Disease, 96(4):, 537–543. https://doi.org/10.1094/PDIS-08-10-0569

Velarde-Félix, S., Garzón-Tiznado, J.-A., Hernández-Verdugo, S., López-Orona, C.-A., & Retes-Manjarrez, J.-E. (2018). Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Canadian Journal of Plant Pathology, 40(2):, 238–247. https://doi.org/10.1080/07060661.2017.1420693

Widyastuti, S.-M., Tasik, S., & Harjono. (2013). Infection process of *Fusarium oxysporum* fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita, 35(2):, 110–118.

393 Table 1 Disease incidence in the commercial nursery fields of *Acacia mangium* forestry in South
394 Sumatra

No.	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1.	Air Sugihan A	500	34	6.8
2.	Air Sugihan B	720	70	9.7~~13.5~~
3.	Air Sugihan C	720	131	18.2
4.	Air Sugihan D	720	266	36.9
5.	Lebong Hitam	604	36	~~5.~~6.0
6.	Air Sugihan F	720	139	19.3

395

396 Table 2 Isolate origin used for pathogenicity test.

Location	Host	Isolate	Number of isolates
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

397

Table 3 Cultural characteristics of *Fusarium oxysporum* obtained from Acacia seedling wilt disease.

Isolate	Colony color	Growth rate (mm/day)
AF01	Top: White to dark pink Bottom: dark pink	11.2 ± 0.6e
AF02	Top: White to pale violet Bottom: Pale violet	10.7 ± 0.6d
AF03	Top: white Bottom: pale violet	10.7 ± 0.5d
AF04	Top: white Bottom: Pale violet	11.1 ± 0.9e
BF05	Top: White to pale violet Bottom: Violet	11.4 ± 0.8e
BF06	Top: White to pale violet Bottom: Violet	7.5 ± 0.7a
BF07	Top: White to dark pink Bottom: Dark pink	7.7 ± 0.6ab
BF08	Top: White to pale violet Bottom: Pale violet	9.0 ± 0.2abc
BF09	Top: White Bottom: Violet to pale violet	8.1 ± 0.6abc
CF10	Top: White to dark pink Bottom: dark pink	9.0 ±1.5bc
DF11	Top: White to dark pink Bottom: dark pink	9.5 ± 0.4cd
DF12	Top: white to pale violet Bottom: pale violet	8.4 ± 0.5abc
DF13	Top: white to pale violet Bottom: pale violet	8.2 ± 0.4abc
EF14	Top: white Bottom: pale violet	9.3 ± 0.8cd
FF15	Top: White Bottom: Violet	11.4 ± 2.1e
LSD 0.05		2.04

The numbers followed by the same letter in the column are not significantly different in the LSD test at 5%.

Table 3~~4~~ Characteristics of the macroscopic and microscopic structure of *Fusarium oxysporum* isolated from infected plants.

Isolate		Microconidia (µm)		Macroconidia (µm)		Chlamydospore (µm)	
		Characteristics	Size (L × W)	Characteristics	Size (L × W)	Characteristics	Size (D)
AF01	Top: White to dark pink Bottom: dark pink	Ellipse–allantoid, 0–1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3–4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Top: White to pale violet Bottom: Pale violet	Oval–reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3–4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Top: white Bottom: pale violet	Ellipse–oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3–4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Top: white Bottom: Pale violet	Ellipse–reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3–4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Top: White to pale violet Bottom: Violet	Ellipse–allantoid, 0–1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3–4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Top: White to pale violet Bottom: Violet	Allantoid shaped, 0–2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3–4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Top: White to dark pink Bottom: Dark pink	Oval–allantoid shaped, 0–2 septa, mostly 0–1septum	7.6 ± 1.8 × 3.5 ± 0.3	3–4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Top: White to pale violet Bottom: Pale violet	Allantoid shaped, 0–2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3–4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Top: White Bottom: Violet to pale violet	Oval–ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3–4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Top: White to dark pink Bottom: dark pink	Ellipse–allantoid, 0–1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3–7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Top: White to dark pink Bottom: dark pink	Ellipse–allantoid, 0–1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3–4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Top: white to pale violet Bottom: pale violet	Ellipse–allantoid shaped, 0–1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3–4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Top: white to pale violet Bottom: pale violet	Ellipse–allantoid, 0–1 septum, mostly 0 septum	10. 3 ± 2.7 × 2.5 ± 0.3	3–4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7
EF14	Top: white Bottom: pale violet	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3–4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Top: White Bottom: Violet	Oval–ellipse, 0–1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3–4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
Mean			8.1 ± 1.7 × 2.8 ± 0.3		37.5 ± 5.6 × 3.9 ± 0.4		7.7 ± 1.0

Formatted: Font: 10 pt
Formatted Table
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt
Formatted: Font: 10 pt

Table 45 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings inoculated with *F. oxysporum*.

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 ab	31.0 ab
AF02	80	2.4 a	46.4 a
AF03	80	1.9 ab	32.6 ab
AF04	80	2.5 a	33.9 ab
BF05	50	2.0 ab	45.5 ab
BF06	100	3.1 a	59.5 a
BF07	60	1.9 ab	32.6 ab
BF08	70	2.1 ab	37.5 ab
BF09	70	1.6 ab	23.6 ab
CF10	90	2.7 a	37.9 a
DF11	90	3.2 a	55.7 a
DF12	80	2.8 a	49.8 a
DF13	80	2.9 a	54.7 a
EF14	60	1.2 ab	15.3 ab
FF15	50	1.6 ab	32.0 ab
Control	0	0 b	0.0 b
P-Value		0.0013	0.0007

The numbers followed by the same letter in the column are not significantly different in the LSD test at 5%.

Fig. 1 Wilt symptoms in the nursery (a) yellowing leaves (b) dry leaves (c).

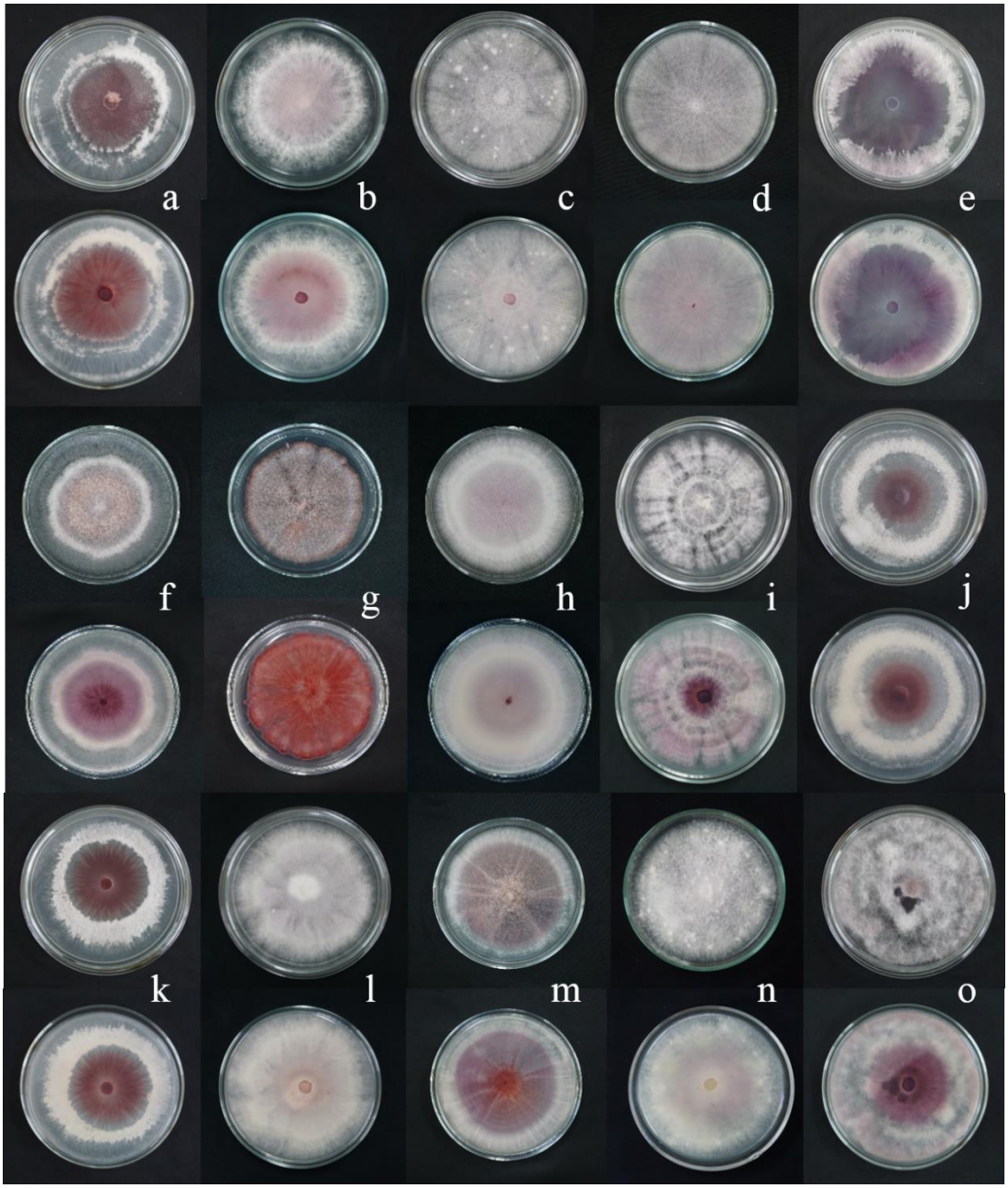
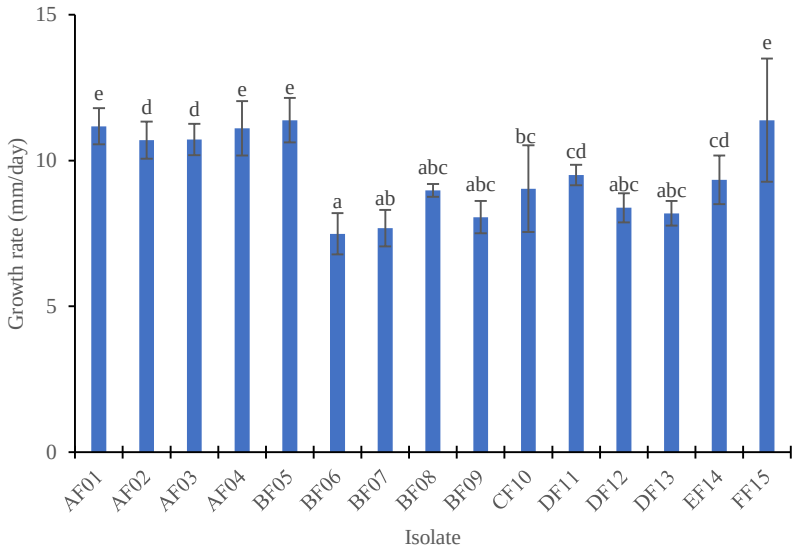


Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f), BF07 (g), BF08 (h), BF09 (i), CF10 (j), DF11 (k), DF12 (l), DF13 (m), EF14 (n), and FF15 (o). Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).



414

415 Fig. 3 Growth rate of *Fusarium oxysporum* from *Acacia mangium* on PDA medium

416

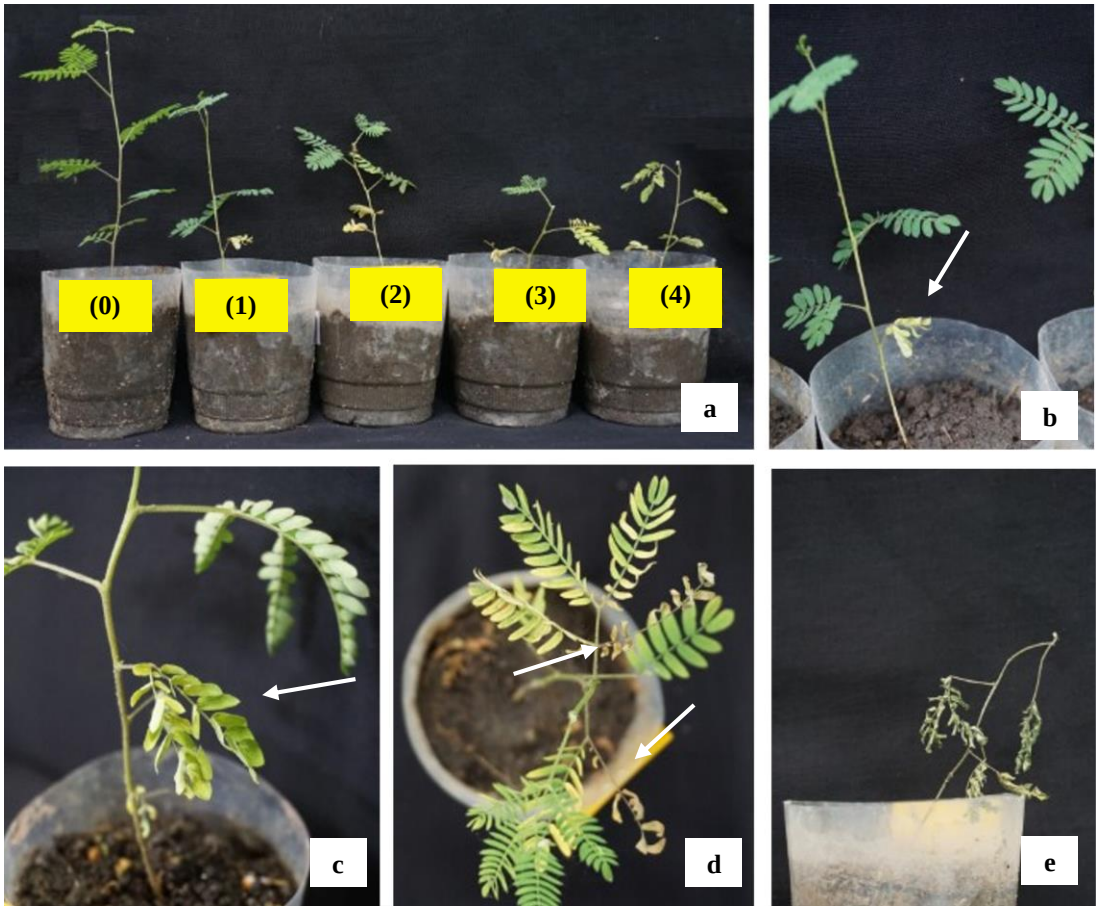
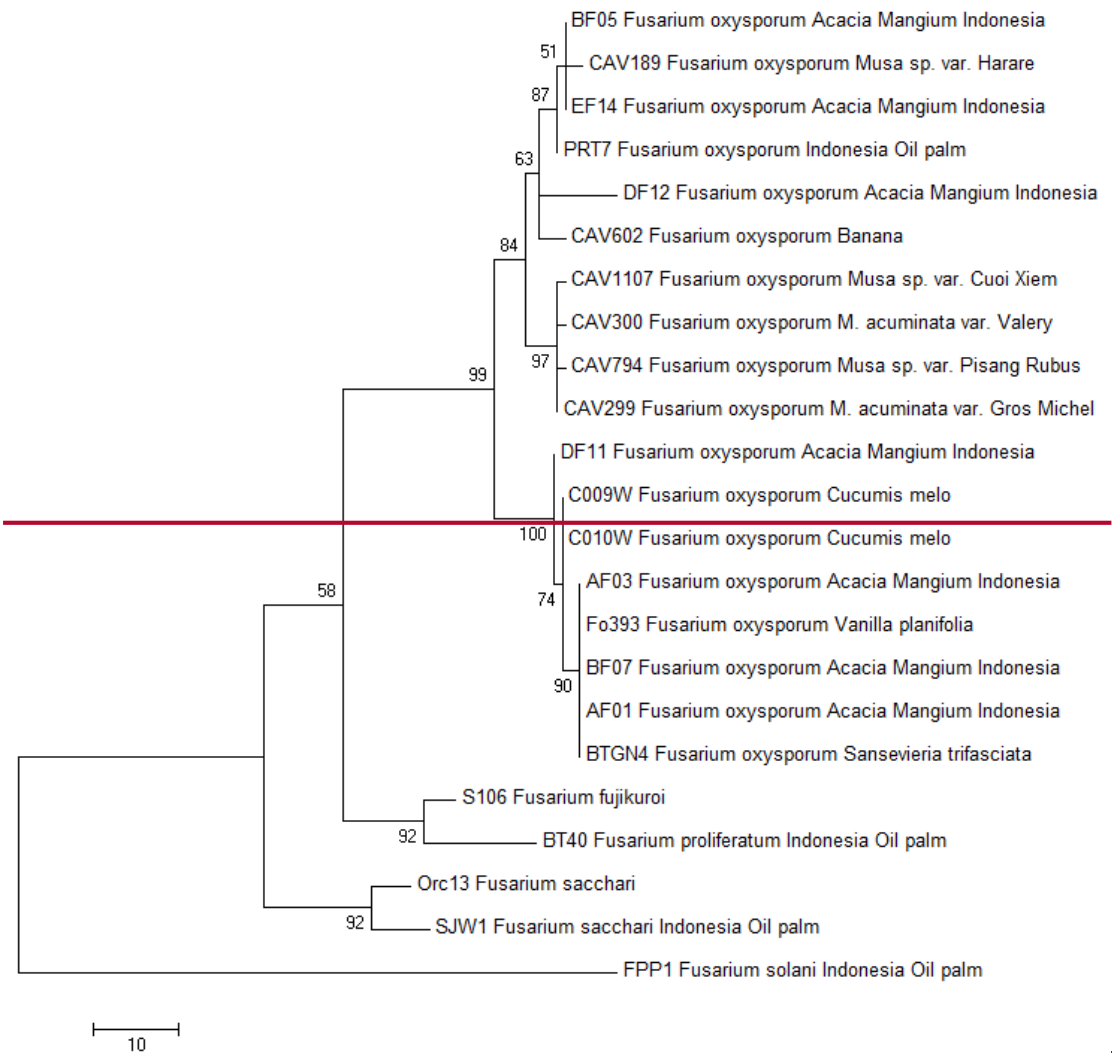


Fig. ~~43~~ Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial symptoms: from lowest leaf, yellowing leaves (b). Advanced symptoms: curved leaves, dry leaves, falling leaves (c,d). and dead plant (e).


BF05 Fusarium oxysporum Acacia Mangium Indonesia
CAV189 Fusarium oxysporum Musa sp. var. Harare
EF14 Fusarium oxysporum Acacia Mangium Indonesia
PRT7 Fusarium oxysporum Indonesia Oil palm
DF12 Fusarium oxysporum Acacia Mangium Indonesia
CAV602 Fusarium oxysporum Banana
CAV1107 Fusarium oxysporum Musa sp. var. Cuoi Xiem
CAV300 Fusarium oxysporum M. acuminata var. Valery
CAV794 Fusarium oxysporum Musa sp. var. Pisang Rubus
CAV299 Fusarium oxysporum M. acuminata var. Gros Michel
DF11 Fusarium oxysporum Acacia Mangium Indonesia
C009W Fusarium oxysporum Cucumis melo
C010W Fusarium oxysporum Cucumis melo
AF03 Fusarium oxysporum Acacia Mangium Indonesia
Fo393 Fusarium oxysporum Vanilla planifolia
BF07 Fusarium oxysporum Acacia Mangium Indonesia
AF01 Fusarium oxysporum Acacia Mangium Indonesia
BTGN4 Fusarium oxysporum Sansevieria trifasciata
S106 Fusarium fujikuroi
BT40 Fusarium proliferatum Indonesia Oil palm
Orc13 Fusarium sacchari
SJW1 Fusarium sacchari Indonesia Oil palm
FPP1 Fusarium solani Indonesia Oil palm
51
87
63
84
97
99
100
74
90
58
92
92
10

421

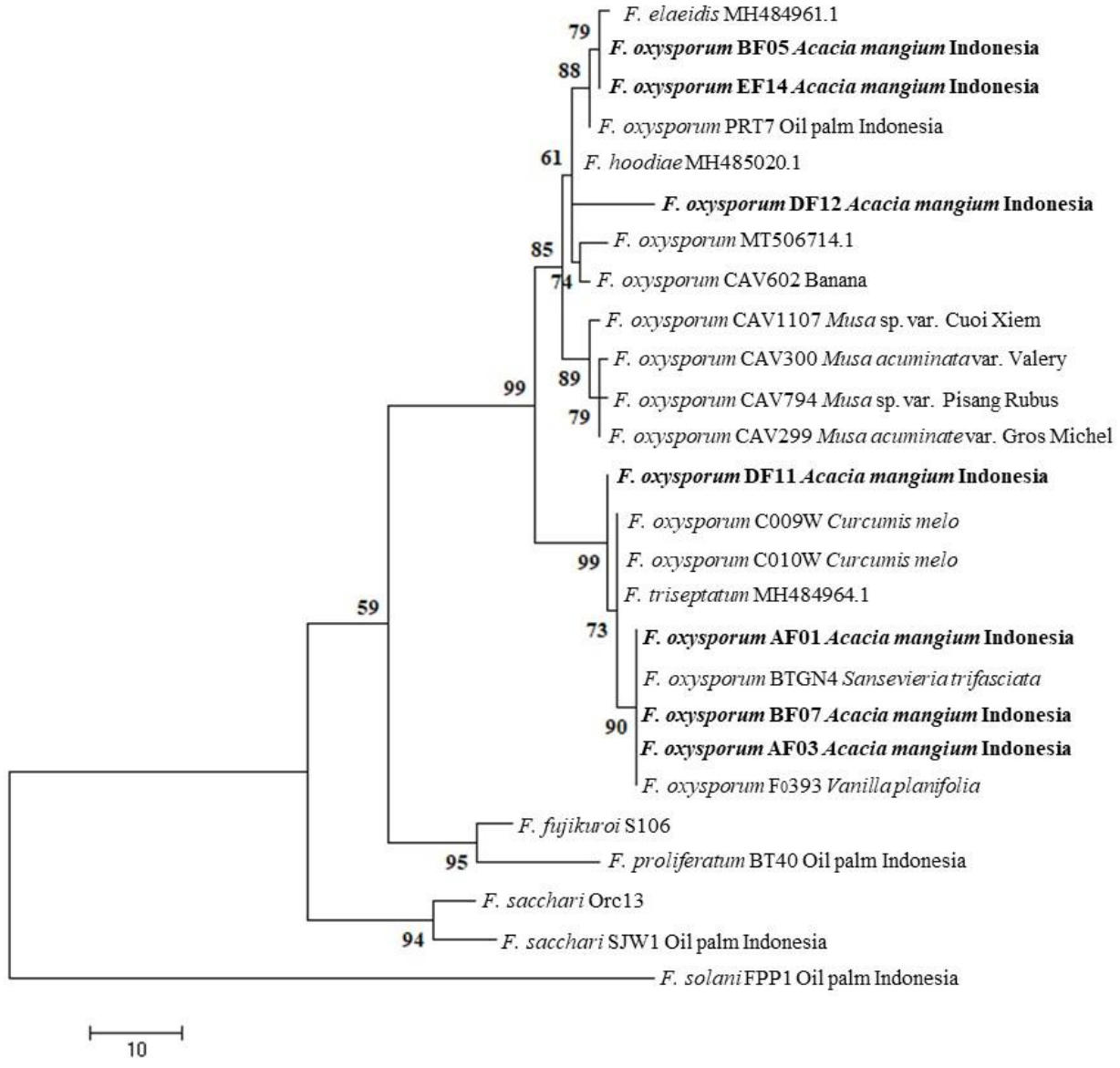


Fig. 54 One out of the ninesix most parsimonious trees showing the genetic relatedness, represented by the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold)(in bold), closely related *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host species and countries of origin are given with the representative isolates. A bootstrap test with 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The genetic distance is indicated by the scale bar.

4.Bukti konfirmasi accepted dan hasil proof corrections (25 April 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

The corrected version of manuscript JFR-D-21-00042, Journal of Forestry Research

4 messages

徐涛 <tao.xv@nefu.edu.cn> Sun, Apr 25, 2021 at 9:48 AM
To: a_muslim@unsri.ac.id

Dr. Ahmad Muslim,

Your submission (JFR-D-21-00042) in the attachment that was just corrected by our language editor from Canada.

Please go through the full text and revise or confirm those which need to be modified again. I wish to receive a confirmed version in four weeks. I will be very glad if you can inform me about you successfully received it.

Thank you for your kind cooperation!

Yours sincerely,

Tao Xu

--

Tao Xu

Editor

Journal of Forestry Research

Northeast Forestry University

26 Hexing Rd., Harbin 150040 , P. R. China

Tel.: +86-451-82191950

JFR-D-21-00042Tao.docx
30783K

a. muslim unsri <a_muslim@unsri.ac.id> Sat, May 1, 2021 at 2:03 PM
To: 徐涛 <tao.xv@nefu.edu.cn>

Dear Prof. Tao Xu

Thank you very much for your kindly corrections to the language of our manuscript. We have revised and some modified the corrections.
I hope I can send you our revision in a few days.

We are really appreciate for your correction and thank you very much

The ~~I~~identification and ~~P~~pathogenicity of *Fusarium oxysporum* causing ~~A~~acacia ~~S~~seedling ~~W~~wilt ~~D~~disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2] Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3] Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

~~Abstract~~

Abstract Wilt disease with unknown etiology causes mass mortality in commercial *Acacia mangium* nurseries~~y~~ ~~of~~in South Sumatra. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; subsequently, the plants wither and die. This research ~~aims to~~ identifies~~y~~ the pathogenic species causing this ~~seedling~~ wilt disease ~~in *A. mangium*~~ and to assess its pathogenicity or virulence. ~~Total 15~~Fifteen isolates of *F. oxysporum* with varying colony sizes and color pigments were recovered from symptomatic *A. mangium* seedlings. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the ~~*Fusarium*~~ pathogen was verified as causing vascular disease. ~~Furthermore,~~ Koch's postulate was~~ere~~ verified~~confirmed~~ by re-isolating the *F. oxysporum* isolates. The ~~P~~pathogen was confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences as *F. oxysporum*.

Keywords: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

Introduction

Black wattle (*Acacia mangium* Willd.) is a flowering tree species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable conditions, this species can grow up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies for its high levels of quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia was 8.67 million hectares, with 81.3% being used for plant cultivation, including *A. mangium*. In addition, in 2017, this species produced the most logs of any other species, reaching 77.5% from a log total of 40,628.8 m^3 (Statistics Indonesia 2018).

A major problem faced in its cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stages of growth and, if ignored, it can spread widely in a plantation.

Fusarium oxysporum Snyder & Hansen is a soil-borne pathogen with a wide host range of hosts and is common in various regions of the world (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019), including forests and industrial plantations (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and regenerative) and survives on plant debris for long periods (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis of the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species

Formatted: Indent: First line: 0 cm

Commented [R1]: You give information from 2018 and then 2017; perhaps reverse this?

is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *A. ~~cacia~~ koa* A. Gray in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980; Dobbs et al. 2020;). Furthermore, *F. oxysporum* was reported to have attacked *A. ~~cacia~~ nilotica* (L.) P. J. H. Hurter & McNabb seedlings in the ~~G~~greenhouse ~~House~~ of the Forest Research Institute, India (Kapoor et al. 2004). ~~On *A. mangium* i~~In Papua (Indonesia), *F. oxysporum* has been found to cause damping-off disease ~~in~~on *A. mangium* seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced ~~stage~~ seedlings of *A. mangium* and the disease's pathogenicity.

Methods and materials ~~ATERIAL AND METHODS~~

Survey and sampling

Soil and diseased plant samples were collected from six commercial, company-owned acacia nurseries~~y estates~~ with a seedling wilt problem. The diseased plants showed initial ~~symptoms in the form of~~ chlorosis; later, the plants ~~went on to~~ wilted and dried~~y~~ up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. ~~Plant s~~Samples showing wilting symptoms were collected and stored in a cool box. ~~during the process.~~ To determine soil infectivity, acacia seeds were sown ~~using~~on a seedbed, and to accelerate germination, they were first soaked in hot water (± 95 ˚C) and left to cool ~~slowly~~ until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 48 h to accelerate the radic~~u~~le development. The germinated seeds were planted on infested field nursery

Formatted: Indent: First line: 0 cm

soil.~~medium.~~ The infected seedlings from the field and the infected~~sted~~ ~~soil~~ nursery soil ~~medium~~ were sampled and the pathogen ~~was also~~ isolated from the plant tissue.

Fungal isolation

Fungi were isolated from the roots of plants growing in the field that showed ~~wilt~~ symptoms of wilt and ~~also~~ from soil infected~~sted~~ with pathogens. ~~Then, t~~The root samples were then washed under running water~~,~~ and the surface ~~was~~ sterilized by dipping ~~them~~ in a solution containing 1% sodium hypochlorite for 2 min, rinsing ~~them~~ three times in~~using sterile~~ distilled water, and drying ~~them~~ on filter paper in a laminar airflow (Suwandi et al. 2012). ~~Then, t~~They were then laid out on ~~planted in~~ a Petri plate of~~containing agar water with~~ 2% (w/v) agar and 0.1% ~~S~~streptomycin~~-~~sulfate, and incubated for 48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium ~~that grew~~ from the root cuttings was transferred to potato dextrose agar (PDA) ~~(Merck, Germany)~~ medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on ~~the~~ Leslie and Summerell (2006)~~.~~ ~~method.~~ The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed ~~based~~ on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia~~,~~ and

Formatted: Indent: First line: 0 cm

Commented [R2]: PDA is quite common so there is no need to specify an origin

Formatted: Indent: First line: 0 cm

macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

~~A pathogenicity~~This test was carried out on *A. mangium* seedlings 30 days after sowing~~. This plant was grown~~ in plastic pots containing 200 g peat soil ~~medium (200 g)~~ which had previously been sterilized~~. using an autoclave.~~ Fungal isolates were grown ~~i~~on a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. This suspension was used as inoculum by pouring 1×10^6 cfu g^{-1} (colony forming unit/g) ~~soil~~ ~~i~~on a soil medium~~,~~ while the uninoculated control was ~~watered only with~~ sterile distilled water. Each isolate was inoculated into the soil of 10 test plants and the experiment ~~was~~ repeated once. Disease incidence was counted ~~by~~as the number of diseased plants out of ~~the~~ 10~~. tested plants.~~ The severity of the disease was calculated for each seedling using a score of 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were ~~then~~ observed ~~for 1~~ over 30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Seven representative~~s~~ fungal isolates with distinct morphological characteristics were selected and grown ~~in a liquid medium of PDB~~ in a cultivation bottle containing 50 mL sterile PDB (200 g

Formatted: Indent: First line: 0 cm

Formatted: Indent: First line: 0 cm

potato; 20 g glucose; 1 L distilled water). ~~s~~Sections of 3–4 ~~-~~ day~~-~~old cultures on the PDA medium, measuring 5 × 5 mm, were placed ~~i~~on a PDB liquid medium and incubated for 3–4 additional~~more~~ days at room temperature. ~~Meanwhile, t~~The fungal mycelium was harvested using vacuum filtration and ~~then it was~~ frozen. ~~The~~ DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA,~~California,~~ USA) following manufacturer's instructions. Its concentration and quality were determined by spectrophotometry using a NanoDrop Spectrophotometry ND‐1000 (NanoDrop Technologies, Montchanin, DE,~~elaware,~~ U.S.A.) and stored at ~~-~~20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ ~~-~~ATGGGTAAGGAAGACAAGAC ~~-~~3′) and EF2 (reverse: 5′~~-~~GGAAGTACCAGTGATCATGTT~~-~~3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio‐rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *A. mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 577 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using

the subtree pruning re-grafting -algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

Results ~~ESULTS AND DISCUSSION~~

~~Results~~

Disease symptoms and wilt incidences

Surveys on seedling wilt were carried out in six acacia nurseries~~y~~ ~~locations~~ ~~in~~of commercial companies~~;, with~~ five sites in the Air Sugihan area and one ~~site~~ in Lebong Hitam (Table 1). The incidence of disease varied between locations from ~~as low as~~ 6.0% up to 36.9%. The early symptoms of seedling wilt started ~~from~~as the lower leaves ~~as they~~ turned yellow~~,, and~~ then black, dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves, which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1). Observations were made by planting acacia seedlings on used soil medium from the commercial nursery, and the results showed that 56.2~~55% of the seedlings~~ were attacked. In the commercial nurseries~~, y,~~ this disease ~~was found to~~ attacks ~~acacia~~ seedlings at an average age of more than 1 month after germination and before the formation of phyllodes or modified petioles or stems.~~false leaves.~~

Formatted: Indent: First line: 0 cm

Commented [R3]:

Morphological characteristics

Fifteen isolates similar to *F. oxysporum* were taken~~isolated~~ from the root tissues of diseased plants (Table 2), and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at ~~a temperature of~~ 27 °C. The PDA cultures ~~on the PDA medium~~ produced ~~several color pigments, such as~~ purple, pale purple and reddish-pink pigments~~,~~ with air hyphae (Fig. 2). These isolates

Formatted: Indent: First line: 0 cm

showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.438 mm/day). BF06 hadshowed the slowest colony growth rate (7.546 mm/day) compared to the others (Fig. 3). They all produced manynumerous microconidia on the false heads of monophialides. Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm, to 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have zero0 to 1one septum, but generally zero.0. The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm, to 39.7 ± 5.9 × 3.8 ± 0.4 µm, and have three3 to seven7 septa, but usuallygenerally 3three. The chlamydospores produced singly or/ in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 3). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

The mMolecular identification achieved by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by making a comparison with those existing in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3–99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.8) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 5). The first clade comprisednsisted of BF05 and EF14, and *F. elaeidis* (MH484961.1) from *Elaeis* spp. The second clade consisted of single isolates from *A. mangium* (DF11), *F. oxysporum* (C009W and C010W) from *Cucumis melo*, and *F. triseptatum* (MH484964.1) from *Ipomoea batatas*. The third clade

Formatted: Indent: First line: 0 cm

575 containedconsisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera*
576 *trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The fourth includedconsisted of
577 DF12 and an isolate of *F. oxysporum* from *Musa* sp. *Tef1* sequences of isolates within third and
578 fourth clade were separated from all 15 cryptic taxa of *F. oxysporum* species complex as described
579 by Lombard et al. (2019).
580
581 ***Pathogenicity tests***
582 *Fusaria*, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A.*
583 *mangium* seedlings. The results showed that the symptom development began with yellowing of
584 the lower leaves or those closest to the base of the stem, , these then their wilting,ed, curvinged
585 upwards, turninged brown to black, drying and , became dry, and fellfalling from the plant. This
586 symptom progresses to the top of the plant, causing it to wither and die. FurthermoreIn addition,
587 infected plants show symptoms of stunted plant growth. The first symptoms appeared 7 to 14 days
588 after inoculation and were followed by total plant collapse within 1 to 10 days. However, some
589 plants showedexperienced complete wilting immediately, without any initial symptoms (Fig. 3).
590 The control plants were healthy and did not show any wilting symptoms. All the isolates were able
591 to infect plants and disease incidence and disease severity were significantly higher compared to
592 the control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease severity and
593 disease progress and their AUDPC values were significantly higher than other isolates and the
594 control (Table 4). With regards toConcerning disease incidence, the *Fusarium* isolates
595 hadpresented a high variation in percentage of disease incidence, ranging from 50−100%. These
596 isolates also induced wilting severities ofranging from 1.2−3.2. The highest disease incidence was
597 caused by isolate BF06 (100%), while the highest disease severity was caused by isolate DF11

(3.2). Based on disease severity, the isolates were grouped into three categories of virulence: high (score 2.0−4.0), moderate (score 1.2−2.0), and low score (0−1.2) virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11 were included in the high virulence group (2.1−3.2), while FF15, BF09, BF07, AF03, and BF05 were in the moderate (1.6−2.0), and only EF14 had a low score. We observed that this pathogen causes vascular disease, where the pathogen could be isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the pathogens were all re-isolated (100%) from infected plants and it was confirmed that the morphological characters of the pathogen were the same as the inoculated *Fusarium* isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *Fusarium oxysporum* has been identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous studies, *F. oxysporum* was reported as the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack germinated acacia seeds, resulting in damping-off, and the identification of this was made solely on morphological characters (Widyastuti et al. 2013). Our research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana* Lamb.

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in

India with diseases severity of 16.986% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85.0% severity (Gardner 1980), and damping-off on *A. mangium* in Papua, Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots, then enter and multiply along the xylem vessels, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

F. oxysporum was ~~determined~~ determined based on morphological identification, both visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the ~~that~~ most similar to the *F. oxysporum* species complex from *Elaeis* sp. (*F. elaeidis*) (Lombard et al. 2019), *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar and Mohd 2019).

Commented [R4]: Should provide the full genus name of each?

~~The f~~Field observation of the disease in the commercial nursery showed that about 36.9% of plants died through *Fusarium* wilt. This attack caused ~~huge~~large economic losses because these nurseries provide~~d~~ seedlings for commercial gardening in South Sumatra ~~totaling~~over 1,324,653 hectares. The high disease incidence in the field was possibly~~probably~~ caused by the previous use of seedlings infe~~c~~sted with the pathogens, which led to rapid development and accumulation through the nursery cycle. This is evident in the high disease incidence on soil media. It is likely

that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt comes from infecsted soil and that pathogens can survive a long ~~time~~periods in the soil, and~~. The pathogens can also survive~~ in other plant debris (Postic et al. 2012; Altinok 2013)

Conclusion

This study ~~presenti~~s the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed through morphological and *tef1* gene sequencing and Koch's postulate. The main source of a primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from ~~a~~seedling soils ~~seedling medium~~ infecsted with pathogens.

Formatted: Indent: First line: 0 cm

Commented [R5]: Infested refers to parasitic diseases caused by animals (mites, ticks, lice); infected refers to disease caused by viruses, bacteria, fungi

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020.

Formatted: Indent: First line: 0 cm

~~REFERENCES~~

References

Altinok HH (2013) Fusarium species isolated from common weeds in eggplant fields and symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161: 335–340.

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150: 609–621.

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4): 166–172.

Dobbs JT, Kim MS, Dudley NS, Klopfenstein NB, Yeh A, Hauff RD, Stewart JE (2020) Whole genome analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) and the development of molecular tools for early detection and monitoring. BMC Genomics 21: 1–15.

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f . sp . *ciceris*. Archives of Phytopathology and Plant Protection 43(2): 174–190.

Edel-Hermann V, Lecomte C (2019) Current status of *Fusarium oxysporum* formae speciales and races. J Phytopathol 109: 512–530.

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp. *koae*, f. sp.

685 nov. J Phytopathol 70(7): 594–597.
686 Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev Phytopathol
687 55: 23–39.
688 Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd . A Fast Growing Tree for Tropical
689 Plantations. J For Sci 29(1): 1–14.
690 Jiménez-Díaz RM, Castillo P, Jiménez-Gasco MdelM, Landa BB, Navas-Cortés JA (2015)
691 Fusarium wilt of chickpeas: Biology, ecology and management. Crop Protection 73: 16–27.
692 Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused by
693 *Fusarium oxysporum*. Journal of Tropical Forest Science 16(4): 453–462.
694 Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of Fusarium
695 species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3): 442–454.
696 Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni M (2016)
697 *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla.
698 Plant Pathol 65(4): 612–625.
699 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis
700 version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870–1874.
701 Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell Publishing, Oxford.
702 Lombard L, Lamprecht SC, Crous PW (2019) Epitypification of *Fusarium oxysporum* – clearing
703 the taxonomic chaos. Persoonia 43: 1-15.
704 Luo X, Yu C (2020) First report of damping - off disease caused by *Fusarium oxysporum* in *Pinus*
705 *massoniana* in China. Journal of Plant Diseases and Protection 127: 401–409.
706 Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium* sp. causing
707 wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. Journal of

Applied Research on Medicinal and Aromatic Plants 100244.

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana : Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Sci. USA 95: 2044–2049.

Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt , focusing on bananas. Appl Soil Ecol 132: 20–33.

Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium species isolated from weeds and plant debris in Croatia. J Phytopathol 160: 76–81.

Statistics Indonesia. (2018). Timber Culture Establishment. BPS-Statistics Indonesia, Jakarta.

Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Protection 117: 77–85.

Suwandi, Akino S, Kondo N (2012) Common spear rot of oil palm in Indonesia. Plant Dis 96(4): 537–543.

Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant Pathol 40(2): 238–247.

Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum* fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2): 110–118.

Fig. 1 (a) ~~W~~wilt symptoms in the nursery, (~~a~~b) yellowing leaves, (~~b~~c) dry leaves ~~(c).~~

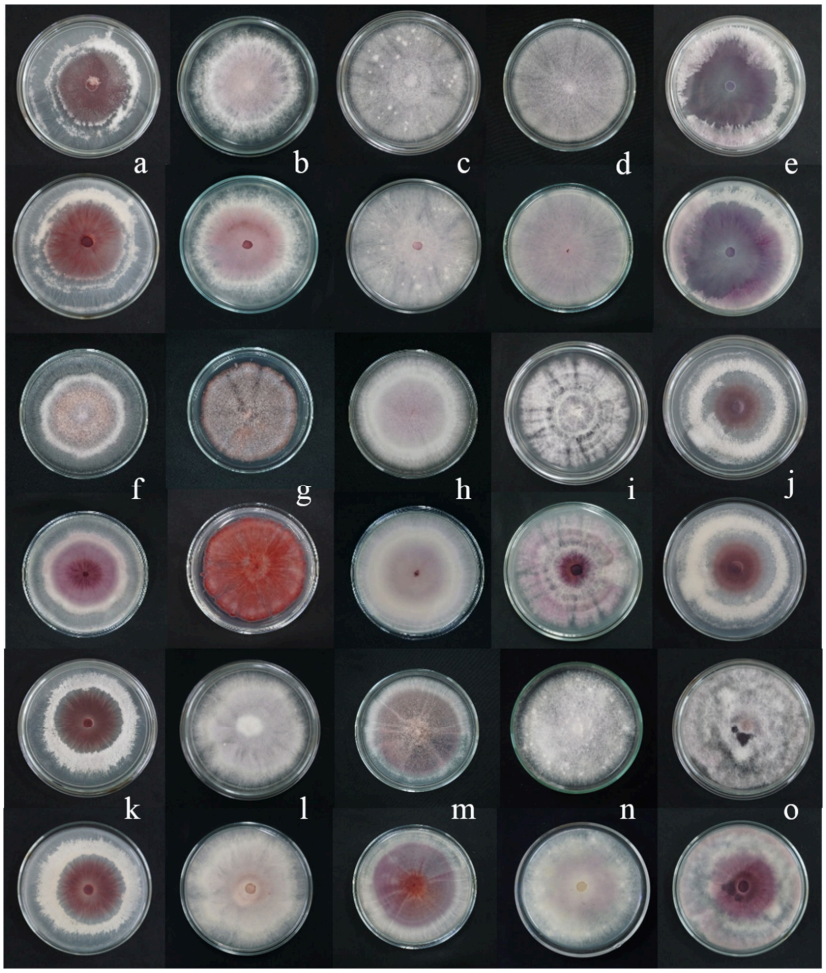
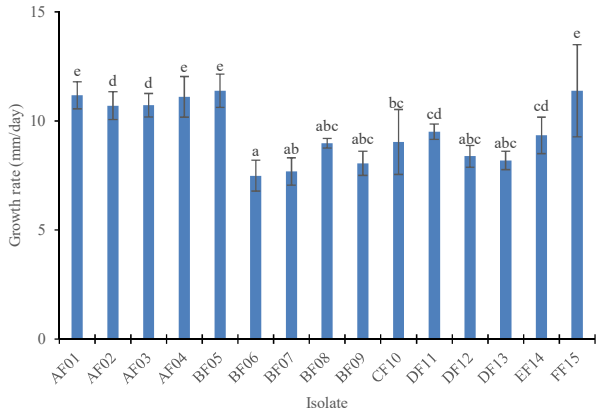


Fig. 2 Colony character on PDA; AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f), BF07 (g), BF08 (h), BF09 (i), CF10 (j), DF11 (k), DF12 (l), DF13 (m), EF14 (n), and FF15 (o). Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).



736

737 Fig. 3 Growth rate of *Fusarium oxysporum* from *Acacia mangium* on PDA medium

738

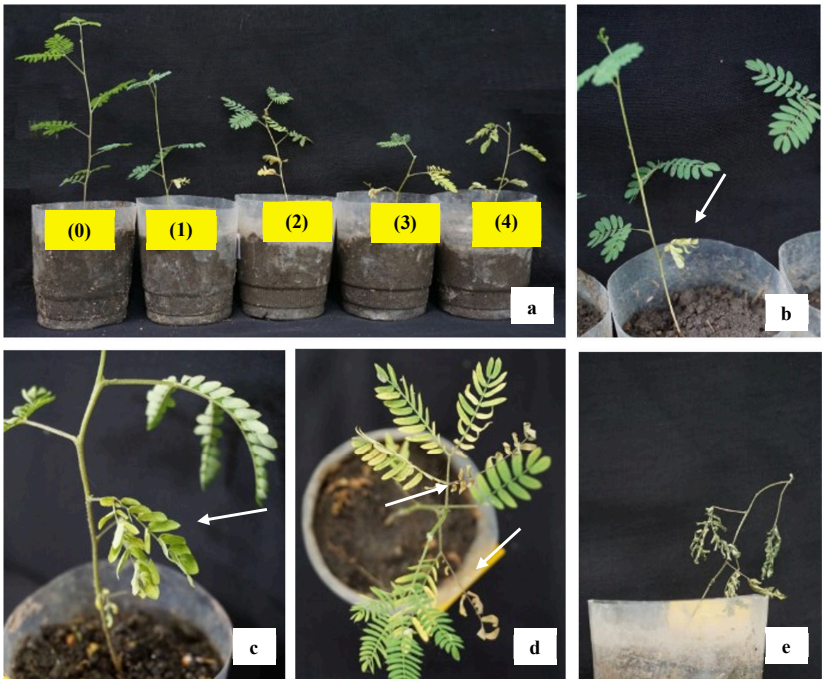
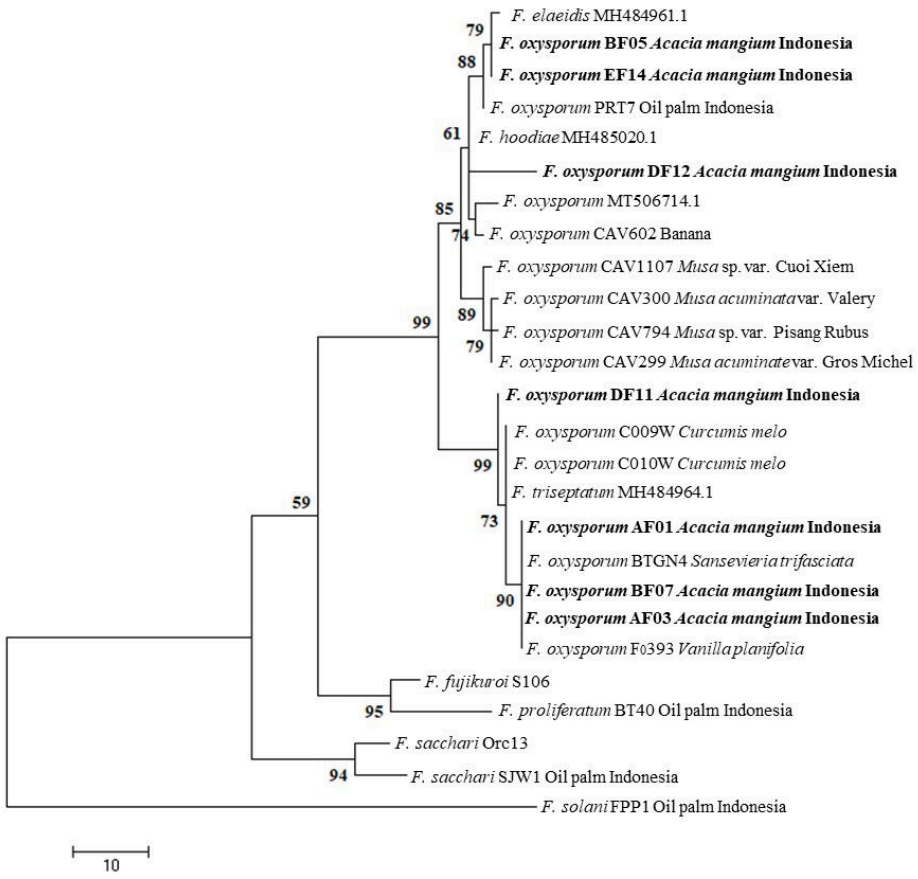


Fig. 4 Disease severity rate: (a) from left: healthy plant to 100% wilted leaves (scale 0-4); (b) initial symptoms: from lowest leaf, yellowing leaves; (c, d) advanced symptoms: curved leaves, dry leaves, falling leaves; (e) dead plant



744

745 Fig. 5 One out of the nine most parsimonious trees showing the genetic relatedness, represented
746 by the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold), closely
747 related *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers,
748 host species and countries of origin are given with the representative isolates. A bootstrap test with
749 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The
750 genetic

Commented [R6]: This is incomplete

751

752 Table 1 Disease incidence in ~~the~~ commercial forest nurseries~~y fields~~ of *Acacia mangium* ~~forestry~~

753 in South Sumatra

No.	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1.	Air Sugihan A	500	34	6.8
2.	Air Sugihan B	720	70	9.7
3.	Air Sugihan C	720	131	18.2
4.	Air Sugihan D	720	266	36.9
5.	Lebong Hitam	604	36	6.0
6.	Air Sugihan F	720	139	19.3

754

Table 2 Isolate origin used for pathogenicity test.

Location	**Host**	**Isolate**	**Number of isolates**
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

757 Table 3 Characteristics of the macroscopic and microscopic structure of *Fusarium oxysporum* isolated from infected plants.

Isolate		Microconidia (μm)		Macroconidia (μm)		Chlamydospore (μm)	
		Characteristics	Size (L × W)	Characteristics	Size (L × W)	Characteristics	Size (D)
AF01	Top: White to dark pink Bottom: dark pink	Ellipse– allantoid, 0– 1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3–4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Top: White to pale violet Bottom: Pale violet	Oval–reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3–4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Top: white Bottom: pale violet	Ellipse–oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3–4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Top: white Bottom: Pale violet	Ellipse–reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3–4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Top: White to pale violet Bottom: Violet	Ellipse– allantoid, 0– 1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3–4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Top: White to pale violet Bottom: Violet	Allantoid shaped, 0–2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3–4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Top: White to dark pink Bottom: Dark pink	Oval– allantoid shaped, 0– 2 septa, mostly 0–1septum	7.6 ± 1.8 × 3.5 ± 0.3	3–4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Top: White to pale violet Bottom: Pale violet	Allantoid shaped, 0–2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3–4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Top: White Bottom: Violet to pale violet	Oval–ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3–4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Top: White to dark pink Bottom: dark pink	Ellipse– allantoid, 0– 1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3–7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Top: White to dark pink Bottom: dark pink	Ellipse– allantoid, 0– 1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3–4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Top: white to pale violet Bottom: pale violet	Ellipse– allantoid shaped, 0– 1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3–4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Top: white to pale violet	Ellipse– allantoid, 0– 1 septum,	10. 3 ± 2.7 × 2.5 ± 0.3	3–4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7

	Bottom: pale violet	mostly 0 septum					
EF14	Top: white Bottom: pale violet	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3–4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Top: White Bottom: Violet	Oval–ellipse, 0–1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3–4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
Mean			8.1 ± 1.7 × 2.8 ± 0.3		37.5 ± 5.6 × 3.9 ± 0.4		7.7 ± 1.0

758

759 Table 4 Incidence, severity and progression of wilt disease in one-month-old acacia
760 seedlings inoculated with *F. oxysporum*.

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 ab	31.0 ab
AF02	80	2.4 a	46.4 a
AF03	80	1.9 ab	32.6 ab
AF04	80	2.5 a	33.9 ab
BF05	50	2.0 ab	45.5 ab
BF06	100	3.1 a	59.5 a
BF07	60	1.9 ab	32.6 ab
BF08	70	2.1 ab	37.5 ab
BF09	70	1.6 ab	23.6 ab
CF10	90	2.7 a	37.9 a
DF11	90	3.2 a	55.7 a
DF12	80	2.8 a	49.8 a
DF13	80	2.9 a	54.7 a
EF14	60	1.2 ab	15.3 ab
FF15	50	1.6 ab	32.0 ab
Control	0	0 b	0.0 b
P-Value		0.0013	0.0007

Commented [R7]: Letters should be superscript ab

761 The numbers followed by the same letter in the column are not significantly different
762 in the LSD test at 5%.
763

5. Bukti konfirmasi submit proof corrections, respon kepada editor, dan artikel yang proof corrections (07 Mei 2021)

Best regard

Ahmad Muslim
Sriwijaya University
[Quoted text hidden]

---

a. muslim unsri <a_muslim@unsri.ac.id> Fri, May 7, 2021 at 7:46 AM
To: 徐涛 <tao.xv@nefu.edu.cn>

May 6, 2021

Dear Prof. Tao Xu
Editor
Journal of Forestry Research

Thank you very much for your kindly corrections to the language of our manuscript. We have revised and make some modified the corrections.

We are really appreciating for your language correction by editor from Canada
Here, we enclose revised version with tracked changes of the manuscript No. JFR-D-21-00042 entitled "The Identification and Pathogenicity of *Fusarium oxysporum* causing Acacia Seedling Wilt Disease" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

Below is a summary of our changes made in response to the language editor comments.

Comment [1]: You give information from 2018 and then 2017; perhaps reverse this?
Our response: It is correct. The data was collected in 2017, but the book was published by Statistic Indonesia in 2018.

Comment [2]: Should provide the full genus name of each?
Our response: Full genus name has been written in result section on line 574, line 575, and line 576.

Comment [3]: Infested refers to parasitic diseases caused by animals (mites, ticks, lice); infected refers to disease caused by viruses, bacteria, fungi.
Our response: The sentence means: soil that contaminated by pathogen inocula, but not soil infected by pathogen. Soil is not a host of pathogen. The word is preferring "contaminated" rather than "infected".

Comment [4]: This is incomplete
Our response: The complete sentence is "The genetic distance is indicated by the scale bar". The changes have been made in the manuscript

Comment [5]: Letters should be superscript ^{ab}
Our response: The changes have been made in the manuscript

We feel that these changes have adequately addressed the comments and suggestions of the language editor. Please feel free to contact me if you need any additional information or clarification.

Thank you very much for your consideration of the manuscript and excellent cooperation

Yours sincerely,
Ahmad Muslim
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id

JFR-D-21-00042TaoRevised.docx
https://drive.google.com/file/d/1VdfszkSNj8YZP_8ufFAo1pa43MFGFfGo/view?usp=sharing

The ~~I~~identification and ~~P~~pathogenicity of *Fusarium oxysporum* causing ~~A~~acacia ~~S~~seedling ~~W~~wilt ~~D~~disease

Soleha Soleha[1], Ahmad Muslim[2*], Suwandi Suwandi[2], Sabaruddin Kadir[3], Rahmat Pratama[1]

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Padang Selasa No. 524, Bukit Besar, Palembang 30139, South Sumatra, Indonesia

[2] Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3] Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

*Corresponding author: a_muslim@unsri.ac.id

~~Abstract~~

Abstract Wilt disease with unknown etiology causes mass mortality in commercial *Acacia mangium* nurseries~~y~~ ~~of~~in South Sumatra. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; subsequently, the plants wither and die. This research ~~aims to~~ identifies~~y~~ the pathogenic species causing this ~~seedling~~ wilt disease ~~in *A. mangium*~~ and to assess its pathogenicity or virulence. ~~Total 15~~Fifteen isolates of *F. oxysporum* with varying colony sizes and color pigments were recovered from symptomatic *A. mangium* seedlings. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the ~~*Fusarium*~~ pathogen was verified as causing vascular disease. ~~Furthermore,~~ Koch's postulate was~~ere~~ verified~~confirmed~~ by re-isolating the *F. oxysporum* isolates. The ~~P~~pathogen was confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences as *F. oxysporum*.

Keywords: *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · pathogenicity

IntroductionNTRODUCTION

Black wattle (*Acacia mangium* Willd.) is a floweringgreen leafy- planttree species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable environmental conditions, this species can grow, up to 30 m with a diameter of 50 cm. It is cultivated on industrial forest plantations by large companies for itsbecause it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia wasere 8.67 million hectares, with 81.30% being used for plant cultivation, including *A. mangium*. Furthermore, In addition, in 2017, this plantspecies produced the most logs of any other species, reaching 77.55% from a log total of 40,628.878 m^3 (Statistics Indonesia 2018).

TheA major problem faced in its cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stages of plant growth and, if ignored, it can spread widely in a plantation.

Fusarium oxysporum *F. oxysporum* Snyder & Hansen is a soil-borne pathogen with a very wide host range of hosts and is common in various regions of the world (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019), including forests and industrial plantations (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and regenerative) and is able to survives on plant debris for a long periods (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis inof the leaves, stunted growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species

Formatted: Indent: First line: 0 cm

Commented [R1]: You give information from 2018 and then 2017; perhaps reverse this?

Commented [W8P2R1]: It is correct. The data was collected in 2017, but the book was published by Statistic Indonesia in 2018.

is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *A. ~~cacia~~ koa* A. Gray in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980; Dobbs et al. 2020;). Furthermore, *F. oxysporum* was reported to have attacked *A. ~~cacia~~ nilotica* (L.) P. J. H. Hurter & McNabb seedlings in the ~~G~~greenhouse ~~House~~ of the Forest Research Institute, India (Kapoor et al. 2004). ~~On *A. mangium* i~~In Papua (Indonesia), *F. oxysporum* has been found to cause damping-off disease ~~in~~on *A. mangium* seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced ~~stage~~ seedlings of *A. mangium* and the disease's pathogenicity.

Methods and materials ~~ATERIAL AND METHODS~~

Survey and sampling

Soil and diseased plant samples were collected from six commercial, company-owned acacia nurseries~~y estates~~ with a seedling wilt problem. The diseased plants showed initial ~~symptoms in the form of~~ chlorosis; later, the plants ~~went on to~~ wilted and dried~~y~~ up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. ~~Plant s~~Samples showing wilting symptoms were collected and stored in a cool box. ~~during the process.~~ To determine soil infectivity, acacia seeds were sown ~~using~~on a seedbed, and to accelerate germination, they were first soaked in hot water (± 95 ˚C) and left to cool ~~slowly~~ until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 48 h to accelerate the radic~~u~~le development. The germinated seeds were planted on infested field nursery

Formatted: Indent: First line: 0 cm

soil.~~medium.~~ The infected seedlings from the field and the infected~~sted~~ ~~soil~~ nursery soil ~~medium~~ were sampled and the pathogen ~~was also~~ isolated from the plant tissue.

Fungal isolation

Fungi were isolated from the roots of plants growing in the field that showed ~~wilt~~ symptoms of wilt and ~~also~~ from soil infected~~sted~~ with pathogens. ~~Then, t~~The root samples were then washed under running water~~,~~ and the surface ~~was~~ sterilized by dipping ~~them~~ in a solution containing 1% sodium hypochlorite for 2 min, rinsing ~~them~~ three times in~~using sterile~~ distilled water, and drying ~~them~~ on filter paper in a laminar airflow (Suwandi et al. 2012). ~~Then, t~~They were then laid out on ~~planted in~~ a Petri plate of~~containing agar water with~~ 2% (w/v) agar and 0.1% ~~S~~streptomycin~~-~~sulfate, and incubated for 48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium ~~that grew~~ from the root cuttings was transferred to potato dextrose agar (PDA) ~~(Merck, Germany)~~ medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on ~~the~~ Leslie and Summerell (2006)~~.~~ ~~method.~~ The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed ~~based~~ on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia~~,~~ and

Formatted: Indent: First line: 0 cm

Commented [R3]: PDA is quite common so there is no need to specify an origin

Formatted: Indent: First line: 0 cm

macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

~~A pathogenicity~~This test was carried out on *A. mangium* seedlings 30 days after sowing~~. This plant was grown~~ in plastic pots containing 200 g peat soil ~~medium (200 g)~~ which had previously been sterilized~~. using an autoclave.~~ Fungal isolates were grown ~~i~~on a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. This suspension was used as inoculum by pouring 1×10^6 cfu g^{-1} (colony forming unit/g) ~~soil~~ ~~i~~on a soil medium~~,~~ while the uninoculated control was ~~watered only with~~ sterile distilled water. Each isolate was inoculated into the soil of 10 test plants and the experiment ~~was~~ repeated once. Disease incidence was counted ~~by~~as the number of diseased plants out of ~~the~~ 10~~. tested plants.~~ The severity of the disease was calculated for each seedling using a score of 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were ~~then~~ observed ~~for 1~~–over 30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Seven representative~~s~~ fungal isolates with distinct morphological characteristics were selected and grown ~~in a liquid medium of PDB~~ in a cultivation bottle containing 50 mL sterile PDB (200 g

Formatted: Indent: First line: 0 cm

Formatted: Indent: First line: 0 cm

potato; 20 g glucose; 1 L distilled water). ~~s~~Sections of 3–4 ~~-~~ day~~-~~old cultures on the PDA medium, measuring 5 × 5 mm, were placed ~~i~~on a PDB liquid medium and incubated for 3–4 additional~~more~~ days at room temperature. ~~Meanwhile, t~~The fungal mycelium was harvested using vacuum filtration and ~~then it was~~ frozen. ~~The~~ DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA,~~California,~~ USA) following manufacturer's instructions. Its concentration and quality were determined by spectrophotometry using a NanoDrop Spectrophotometry ND‐1000 (NanoDrop Technologies, Montchanin, DE,~~elaware,~~ U.S.A.) and stored at ~~-~~20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′ ~~-~~ATGGGTAAGGAAGACAAGAC ~~-~~3′) and EF2 (reverse: 5′~~-~~GGAAGTACCAGTGATCATGTT~~-~~3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio‐rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium oxysporum* from *A. mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 577 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using

the subtree pruning re-grafting -algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

Results ~~ESULTS AND DISCUSSION~~

~~Results~~

Disease symptoms and wilt incidences

Surveys on seedling wilt were carried out in six acacia nurseries~~y~~ ~~locations~~ ~~in~~of commercial companies~~;, with~~ five sites in the Air Sugihan area and one ~~site~~ in Lebong Hitam (Table 1). The incidence of disease varied between locations from ~~as low as~~ 6.0% up to 36.9%. The early symptoms of seedling wilt started ~~from~~as the lower leaves ~~as they~~ turned yellow~~,, and~~ then black, dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves, which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1). Observations were made by planting acacia seedlings on used soil medium from the commercial nursery, and the results showed that 56.2~~55% of the seedlings~~ were attacked. In the commercial nurseries~~, y,~~ this disease ~~was found to~~ attacks ~~acacia~~ seedlings at an average age of more than 1 month after germination and before the formation of phyllodes or modified petioles or stems.~~false leaves.~~

Formatted: Indent: First line: 0 cm

Commented [R4]:

Morphological characteristics

Fifteen isolates similar to *F. oxysporum* were taken~~isolated~~ from the root tissues of diseased plants (Table 2), and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at ~~a temperature of~~ 27 °C. The PDA cultures ~~on the PDA medium~~ produced ~~several color pigments, such as~~ purple, pale purple and reddish-pink pigments~~,~~ with air hyphae (Fig. 2). These isolates

Formatted: Indent: First line: 0 cm

showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.438 mm/day). BF06 hadshowed the slowest colony growth rate (7.546 mm/day) compared to the others (Fig. 3). They all produced manynumerous microconidia on the false heads of monophialides. Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm, to 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have zero0 to 1one septum, but generally zero.0. The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm, to 39.7 ± 5.9 × 3.8 ± 0.4 µm, and have three3 to seven7 septa, but usuallygenerally 3three. The chlamydospores produced singly or/ in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 3). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

The mMolecular identification achieved by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by making a comparison with those existing in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3–99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.8) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 5). The first clade comprisednsisted of BF05 and EF14, and *F. elaeidis* (MH484961.1) from *Elaeis* spp. The second clade consisted of single isolates from *A. mangium* (DF11), *F. oxysporum* (C009W and C010W) from *Cucumis melo*, and *F. triseptatum* (MH484964.1) from *Ipomoea batatas*. The third clade

Formatted: Indent: First line: 0 cm

575 containedconsisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera*
576 *trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The fourth includedconsisted of
577 DF12 and an isolate of *F. oxysporum* from *Musa* sp. *Tef1* sequences of isolates within third and
578 fourth clade were separated from all 15 cryptic taxa of *F. oxysporum* species complex as described
579 by Lombard et al. (2019).
580
581 ***Pathogenicity tests***
582 *Fusaria*, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A.*
583 *mangium* seedlings. The results showed that the symptom development began with yellowing of
584 the lower leaves or those closest to the base of the stem, , these then their wilting,ed, curvinged
585 upwards, turninged brown to black, drying and , became dry, and fellfalling from the plant. This
586 symptom progresses to the top of the plant, causing it to wither and die. FurthermoreIn addition,
587 infected plants show symptoms of stunted plant growth. The first symptoms appeared 7 to 14 days
588 after inoculation and were followed by total plant collapse within 1 to 10 days. However, some
589 plants showedexperienced complete wilting immediately, without any initial symptoms (Fig. 3).
590 The control plants were healthy and did not show any wilting symptoms. All the isolates were able
591 to infect plants and disease incidence and disease severity were significantly higher compared to
592 the control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease severity and
593 disease progress and their AUDPC values were significantly higher than other isolates and the
594 control (Table 4). With regards toConcerning disease incidence, the *Fusarium* isolates
595 hadpresented a high variation in percentage of disease incidence, ranging from 50−100%. These
596 isolates also induced wilting severities ofranging from 1.2−3.2. The highest disease incidence was
597 caused by isolate BF06 (100%), while the highest disease severity was caused by isolate DF11

(3.2). Based on disease severity, the isolates were grouped into three categories of virulence: high (score 2.0−4.0), moderate (score 1.2−2.0), and low score (0−1.2) virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11 were included in the high virulence group (2.1−3.2), while FF15, BF09, BF07, AF03, and BF05 were in the moderate (1.6−2.0), and only EF14 had a low score. We observed that this pathogen causes vascular disease, where the pathogen could be isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the pathogens were all re-isolated (100%) from infected plants and it was confirmed that the morphological characters of the pathogen were the same as the inoculated *Fusarium* isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *Fusarium oxysporum* has been identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous studies, *F. oxysporum* was reported as the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack germinated acacia seeds, resulting in damping-off, and the identification of this was made solely on morphological characters (Widyastuti et al. 2013). Our research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana* Lamb.

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in

India with diseases severity of 16.986% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85.0% severity (Gardner 1980), and damping-off on *A. mangium* in Papua, Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots, then enter and multiply along the xylem vessels, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

F. oxysporum was ~~determined~~ determined based on morphological identification, both visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the ~~that~~ most similar to the *F. oxysporum* species complex from *Elaeis* sp. (*F. elaeidis*) (Lombard et al. 2019), *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar and Mohd 2019).

~~The f~~Field observation of the disease in the commercial nursery showed that about 36.9% of plants died through *Fusarium* wilt. This attack caused ~~huge~~large economic losses because these nurseries provide~~d~~ seedlings for commercial gardening in South Sumatra ~~totaling~~over 1,324,653 hectares. The high disease incidence in the field was possibly~~probably~~ caused by the previous use of seedlings infe~~c~~sted with the pathogens, which led to rapid development and accumulation through the nursery cycle. This is evident in the high disease incidence on soil media. It is likely

Commented [R5]: Should provide the full genus name of each?

Commented [W8P6R5]: Full genus name has been written in result section on line 574, line 575, and line 576.

that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt comes from infecsted soil and that pathogens can survive a long ~~time~~periods in the soil, and~~. The pathogens can also survive~~ in other plant debris (Postic et al. 2012; Altinok 2013)

Conclusion

This study ~~presenti~~s the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed through morphological and *tef1* gene sequencing and Koch's postulate. The main source of a primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from ~~a~~seedling soils ~~seedling medium~~ infecsted with pathogens.

Acknowledgement

This research was funded by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020-2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/UN9/ SB3.LP2M.PT/2020.

Formatted: Indent: First line: 0 cm

Commented [R7]: Infested refers to parasitic diseases caused by animals (mites, ticks, lice); infected refers to disease caused by viruses, bacteria, fungi

Commented [W8P8R7]: The sentence means: soil that contaminated by pathogen inocula, but not soil infected by pathogen. Soil is not a host of pathogen. The word is prefer "contaminated" rather than "infected".

Formatted: Indent: First line: 0 cm

~~REFERENCES~~

References

Altinok HH (2013) Fusarium species isolated from common weeds in eggplant fields and symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161: 335–340.

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150: 609–621.

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished.

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4): 166–172.

Dobbs JT, Kim MS, Dudley NS, Klopfenstein NB, Yeh A, Hauff RD, Stewart JE (2020) Whole genome analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) and the development of molecular tools for early detection and monitoring. BMC Genomics 21: 1–15.

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f . sp . *ciceris*. Archives of Phytopathology and Plant Protection 43(2): 174–190.

Edel-Hermann V, Lecomte C (2019) Current status of *Fusarium oxysporum* formae speciales and races. J Phytopathol 109: 512–530.

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp. *koae*, f. sp.

685 nov. J Phytopathol 70(7): 594–597.
686 Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev Phytopathol
687 55: 23–39.
688 Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd . A Fast Growing Tree for Tropical
689 Plantations. J For Sci 29(1): 1–14.
690 Jiménez-Díaz RM, Castillo P, Jiménez-Gasco MdelM, Landa BB, Navas-Cortés JA (2015)
691 Fusarium wilt of chickpeas: Biology, ecology and management. Crop Protection 73: 16–27.
692 Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused by
693 *Fusarium oxysporum*. Journal of Tropical Forest Science 16(4): 453–462.
694 Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of Fusarium
695 species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3): 442–454.
696 Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni M (2016)
697 *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla.
698 Plant Pathol 65(4): 612–625.
699 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis
700 version 7.0 for bigger datasets. Mol Biol Evol 33(7): 1870–1874.
701 Leslie JF, Summerell BA (2006) The Fusarium Laboratory Manual. Blackwell Publishing, Oxford.
702 Lombard L, Lamprecht SC, Crous PW (2019) Epitypification of *Fusarium oxysporum* – clearing
703 the taxonomic chaos. Persoonia 43: 1-15.
704 Luo X, Yu C (2020) First report of damping - off disease caused by *Fusarium oxysporum* in *Pinus*
705 *massoniana* in China. Journal of Plant Diseases and Protection 127: 401–409.
706 Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium* sp. causing
707 wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. Journal of

Applied Research on Medicinal and Aromatic Plants 100244.

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana : Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Sci. USA 95: 2044–2049.

Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt , focusing on bananas. Appl Soil Ecol 132: 20–33.

Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium species isolated from weeds and plant debris in Croatia. J Phytopathol 160: 76–81.

Statistics Indonesia. (2018). Timber Culture Establishment. BPS-Statistics Indonesia, Jakarta.

Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Protection 117: 77–85.

Suwandi, Akino S, Kondo N (2012) Common spear rot of oil palm in Indonesia. Plant Dis 96(4): 537–543.

Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant Pathol 40(2): 238–247.

Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum* fungus: A cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2): 110–118.

Fig. 1 (a) ~~W~~wilt symptoms in the nursery, (~~a~~b) yellowing leaves, (~~b~~c) dry leaves ~~(c).~~

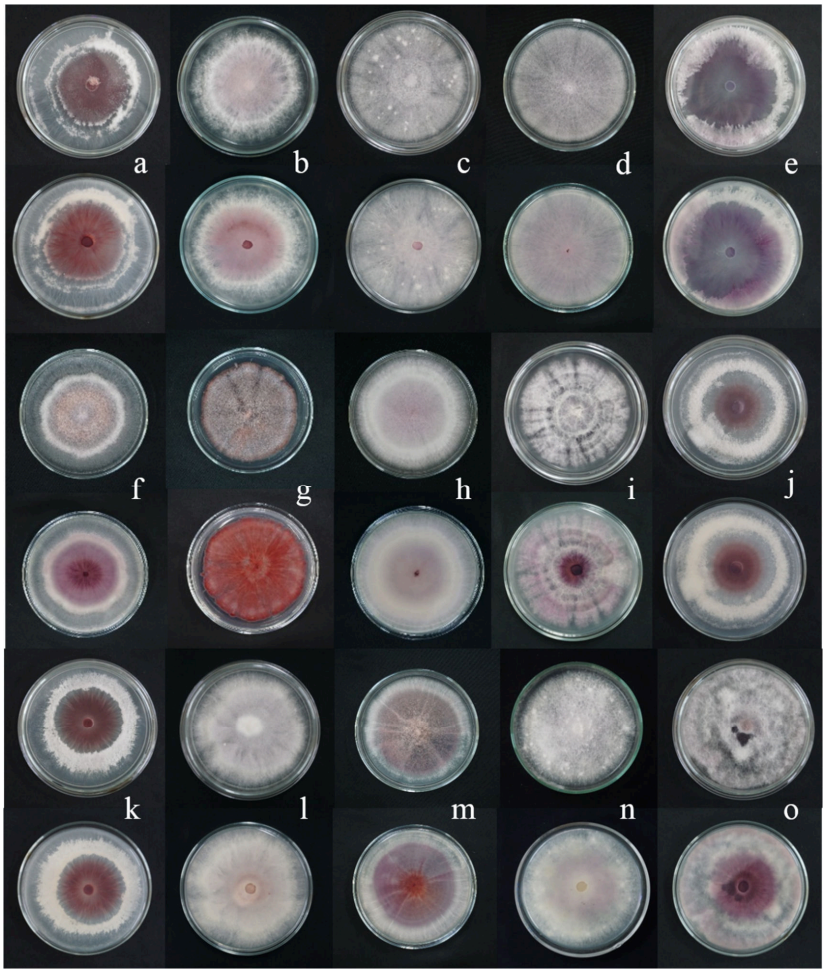
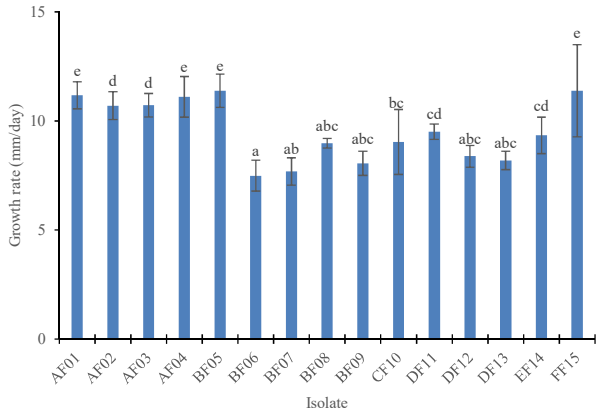


Fig. 2 Colony character on PDA;~~:~~ (a) AF01~~(a)~~, (b)AF02~~(b)~~, (c) AF03~~(c)~~, (d) AF04~~(d)~~, (e) BF05 ~~(e)~~, (f) BF06~~(f)~~, (g) BF07~~(g)~~, (h) BF08~~(h)~~, (i) BF09~~(i)~~, (j) CF10~~(j)~~, (k) DF11~~(k)~~, (l) DF12~~(l)~~, (m) DF13~~(m)~~, (n) EF14~~(n)~~, and (o) FF15~~(o)~~. (1st, 3rd, and 5th line) Colony on top surface ~~(1st, 3rd, and 5th line)~~, (2nd, 4th, and 6th line) Colony on bottom surface ~~(2nd, 4th, and 6th line)~~.



737

738 Fig. 3 Growth rate of *Fusarium oxysporum* from *Acacia mangium* on PDA medium

739

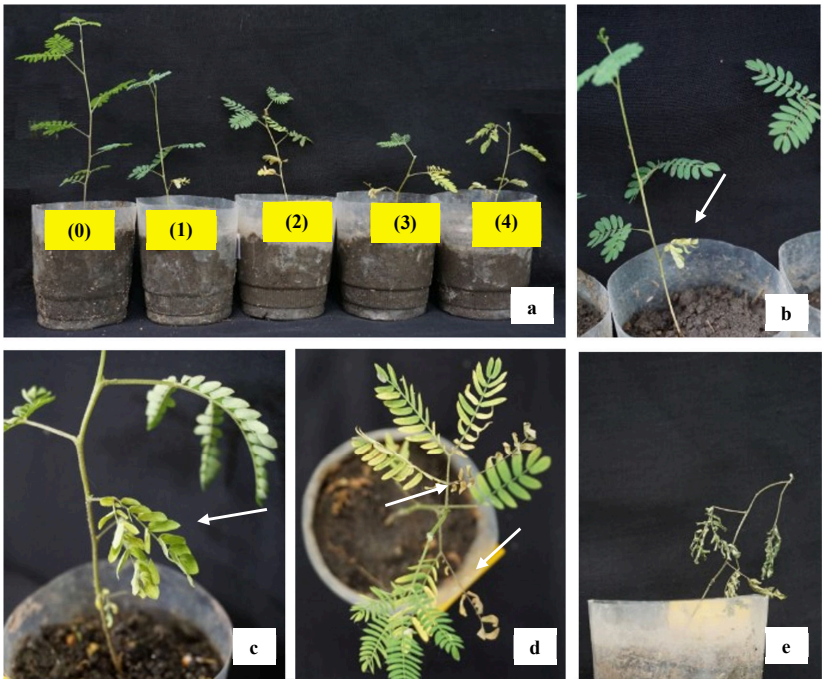


Fig. 4 Disease severity rate: ~~.~~ (a) ~~F~~from left: healthy plant to 100% wilted leaves (scale 0-4); ~~(a).~~ (b) ~~I~~initial symptoms: from lowest leaf, yellowing leaves; ~~(b).~~(c, d) ~~A~~advanced symptoms: curved leaves, dry leaves, falling leaves; ~~(c,d). and~~(e) dead plant ~~(e).~~

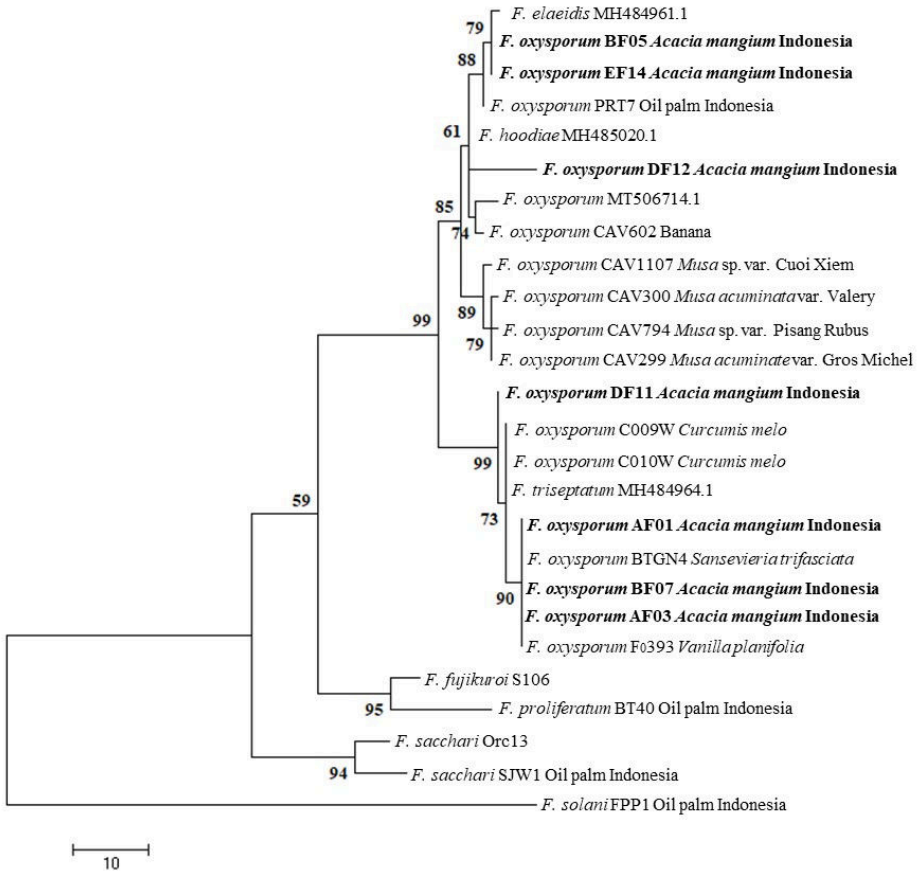


Fig. 5 One out of the nine most parsimonious trees showing the genetic relatedness, represented by the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold), closely related *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host species and countries of origin are given with the representative isolates. A bootstrap test with 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The genetic distance is indicated by the scale bar.

Commented [R9]: This is incomplete

Commented [W8P10R9]: The complete sentence is "The genetic distance is indicated by the scale bar".

753 Table 1 Disease incidence in ~~the~~ commercial forest nurseries~~y fields~~ of *Acacia mangium* ~~forestry~~

754 in South Sumatra

No.	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1.	Air Sugihan A	500	34	6.8
2.	Air Sugihan B	720	70	9.7
3.	Air Sugihan C	720	131	18.2
4.	Air Sugihan D	720	266	36.9
5.	Lebong Hitam	604	36	6.0
6.	Air Sugihan F	720	139	19.3

755

Table 2 Isolate origin used for pathogenicity test.

Location	**Host**	**Isolate**	**Number of isolates**
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

Table 3 Characteristics of the macroscopic and microscopic structure of *Fusarium oxysporum* isolated from infected plants

Isolate		Microconidia (μm)		Macroconidia (μm)		Chlamydospore (μm)	
		Characteristics	Size (L × W)	Characteristics	Size (L × W)	Characteristics	Size (D)
AF01	Top: White to dark pink Bottom: dark pink	Ellipse– allantoid, 0– 1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3–4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Top: White to pale violet Bottom: Pale violet	Oval–reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3–4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Top: white Bottom: pale violet	Ellipse–oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3–4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Top: white Bottom: Pale violet	Ellipse–reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3–4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Top: White to pale violet Bottom: Violet	Ellipse– allantoid, 0– 1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3–4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Top: White to pale violet Bottom: Violet	Allantoid shaped, 0–2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3–4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Top: White to dark pink Bottom: Dark pink	Oval– allantoid shaped, 0– 2 septa, mostly 0–1septum	7.6 ± 1.8 × 3.5 ± 0.3	3–4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Top: White to pale violet Bottom: Pale violet	Allantoid shaped, 0–2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3–4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Top: White Bottom: Violet to pale violet	Oval–ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3–4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Top: White to dark pink Bottom: dark pink	Ellipse– allantoid, 0– 1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3–7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Top: White to dark pink Bottom: dark pink	Ellipse– allantoid, 0– 1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3–4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Top: white to pale violet Bottom: pale violet	Ellipse– allantoid shaped, 0– 1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3–4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Top: white to pale violet	Ellipse– allantoid, 0– 1 septum,	10. 3 ± 2.7 × 2.5 ± 0.3	3–4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7

	Bottom: pale violet	mostly 0 septum					
EF14	Top: white Bottom: pale violet	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3–4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Top: White Bottom: Violet	Oval–ellipse, 0–1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3–4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
Mean			8.1 ± 1.7 × 2.8 ± 0.3		37.5 ± 5.6 × 3.9 ± 0.4		7.7 ± 1.0

Table 4 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings inoculated with *F. oxysporum*.

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 [ab]	31.0 [ab]
AF02	80	2.4 [a]	46.4 [a]
AF03	80	1.9 [ab]	32.6 [ab]
AF04	80	2.5 [a]	33.9 [ab]
BF05	50	2.0 [ab]	45.5 [ab]
BF06	100	3.1 [a]	59.5 [a]
BF07	60	1.9 [ab]	32.6 [ab]
BF08	70	2.1 [ab]	37.5 [ab]
BF09	70	1.6 [ab]	23.6 [ab]
CF10	90	2.7 [a]	37.9 [a]
DF11	90	3.2 [a]	55.7 [a]
DF12	80	2.8 [a]	49.8 [a]
DF13	80	2.9 [a]	54.7 [a]
EF14	60	1.2 [ab]	15.3 [ab]
FF15	50	1.6 [ab]	32.0 [ab]
Control	0	0 [b]	0.0 [b]
P-Value		0.0013	0.0007

The numbers followed by the same letter in the column are not significantly different in the LSD test at 5%.

Commented [R11]: Letters should be superscript [ab]

Commented [W8P12R11]:

Formatted: Superscript

6.Bukti persetujuan terbit jurnal (30 Mei 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Next steps for publishing your article (10.1007/s11676-021-01355-3) - Your publishing agreement

1 message

do-not-reply@springernature.com <do-not-reply@springernature.com> Sun, May 30, 2021 at 2:14 PM
To: a_muslim@unsri.ac.id

SPRINGER NATURE

Dear Dr. Ahmad Muslim ,

Thank you for completing the publishing agreement for your article in Journal of Forestry Research, providing Springer Nature with permission to publish and agreeing to how your manuscript can be used by others.

We have attached a copy of the agreement for your records.

With kind regards,

Springer Nature Author Service

If you have any questions, please do not hesitate to contact our Author Service team at ASJournals@springernature.com

PublicationAgreement.pdf
62K

7. Bukti konfirmasi artikel published online (16 Juli 2021)

a. muslim unsri <a_muslim@unsri.ac.id>

Your article in Journal of Forestry Research is now online

1 message

do-not-reply@springernature.com <do-not-reply@springernature.com> Fri, Jul 16, 2021 at 12:26 AM
To: a_muslim@unsri.ac.id

SPRINGER NATURE

Congratulations

Dear Ahmad Muslim

We are pleased to inform you that your article has just been published:

Title

The identification and pathogenicity of Fusarium oxysporum causing acacia seedling wilt disease

Journal

Journal of Forestry Research

DOI

10.1007/s11676-021-01355-3

Publication Date

2021-07-15

Your article is available online here https://doi.org/10.1007/s11676-021-01355-3 or as a PDF here https://link.springer.com/content/pdf/10.1007/s11676-021-01355-3.pdf.

How can I share my article?

Your final article (Version of Record) may be shared anywhere, at any time, by you or by anyone providing they observe the terms of CC BY and credit you as author, as described above.

So whether you want to share your article on a website, a scholarly collaboration network, by email, on social media, in teaching, or anywhere else, you're free to do so!

Announce your publication

We encourage you to forward this email to your co-authors. Additionally we recommend you mention your article's publication and its DOI on your website or your social media profiles.

Citation Information

Your article can be cited by its DOI 10.1007/s11676-021-01355-3 in the following form:
Author, Journal Title, Year, DOI

Author services to amplify your research

From video abstracts to article metrics to research data support, find out about the author services and how these could benefit you and your research.
Read more at https://www.springernature.com/gp/authors/campaigns/keep-research-in-motion

Thank you again for publishing with Springer Nature. We look forward to your future contributions!

With kind regards,

Springer Nature Author Service

Did you know?
If you are using an ORCID iD and allow for auto-update, your article will appear in your ORCID record automatically. ORCID iDs create a unique, persistent and internationally recognized identifier that attaches your identity to your work, such as articles and their citations. More on ORCID benefits at https://www.springernature.com/gp/researchers/orcid.

If you have any questions, please please check our support portal at https://support.springernature.com/en/support/home or contact our Author Service team at ASJournals@springernature.com

J. For. Res. (2022) 33:711–719
https://doi.org/10.1007/s11676-021-01355-3

ORIGINAL PAPER

The identification and pathogenicity of *Fusarium oxysporum* causing acacia seedling wilt disease

Soleha Soleha[1] · Ahmad Muslim[2] · Suwandi Suwandi[2] · Sabaruddin Kadir[3] · Rahmat Pratama[1]

Received: 23 February 2021 / Accepted: 15 April 2021 / Published online: 15 July 2021

Abstract Wilt disease with unknown etiology causes mass mortality in commercial *Acacia mangium* nurseries in South Sumatra. This pathogen induces symptoms of chlorosis in the lower leaves and develops into the shoots; subsequently, the plants wither and die. This research identifies the pathogenic species causing this wilt disease and to assess its pathogenicity or virulence. Fifteen isolates of *Fusarium oxysporum* with varying colony sizes and color pigments were recovered from symptomatic *A. mangium* seedlings. The pathogenicity test showed that all isolates could infect plants with wilt severity reaching 80%, and the pathogen was verified as causing vascular disease. Koch's postulate was verified by re-isolating the *F. oxysporum* isolates. The pathogen was confirmed by observing the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences as *F. oxysporum*.

Keywords *Acacia mangium* · *Fusarium oxysporum* · Seedling wilt · Pathogenicity

Introduction

Black wattle (*Acacia mangium* Willd.) is a flowering tree species native to Papua, West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in Australia. Under favorable conditions, this species can grow up to 30 m with a diameter of 50 cm. It is cultivated on industrial plantations by large companies for its high levels of quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the total land area controlled by industrial plantation forest companies in Indonesia was 8.67 million hectares, with 81.3% used for plant cultivation, including *A. mangium*. In addition, in 2017, this species produced the most logs of any other species, reaching 77.5% from a log total of 40,628.8 m^3 (Statistics Indonesia 2018).

A major problem in its cultivation is an unknown cause of seedling wilt disease. It occurs during the initial stages of growth and if ignored, can spread widely in a plantation.

Fusarium oxysporum Snyder and Hansen is a soil-borne pathogen with a wide range of hosts and is common in various regions of the world (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann and Lecomte 2019), including forests and industrial plantations (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and regenerative) and survives on plant debris for long periods (Postic et al. 2012; Meena and Roy 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The symptoms involve chlorosis of the leaves, stunted

Project funding: The work was supported by the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education through the PMDSU scholarship 2020−2021 according to the Director of Research and Community Service, Directorate of Research and Community Service, chaired by Ahmad Muslim number 0124/ UN9/ SB3.LP2M.PT/2020.

The online version is available at http://www.springerlink.com.

Corresponding editor: Tao Xu.

✉ Ahmad Muslim
a_muslim@unsri.a.id

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya, Jl. Padang Selasa No. 524, Bukit Besar, Palembang, South Sumatra 30139, Indonesia

[2] Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

[3] Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia

growth, discoloration of the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

Previous studies have reported seedling wilt disease affecting *A. koa* A. Gray in Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980; Dobbs et al. 2020). Furthermore, *F. oxysporum* was reported to have attacked *A. nilotica* (L.) P. J. H. Hurter & McNabb seedlings in the greenhouse of the Forest Research Institute, India (Kapoor et al. 2004). In Papua (Indonesia), *F. oxysporum* has been found to cause damping-off disease on *A. mangium* seedlings six days after germination (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing wilt disease in advanced seedlings of *A. mangium* and the disease's pathogenicity.

Materials and methods

Survey and sampling

Soil and diseased plant samples were collected from six commercial, company-owned acacia nurseries with a seedling wilt problem. The diseased plants showed initial chlorosis; later, the plants wilted and dried up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Samples showing wilting symptoms were collected and stored in a cool box. To determine soil infectivity, acacia seeds were sown on a seedbed, and to accelerate germination, they were first soaked in hot water (± 95 °C) and left to cool until they reached room temperature (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were then incubated for 48 h to accelerate the radicle development. The germinated seeds were planted on infested field nursery soil. The infected seedlings from the field and the infected nursery soil were sampled and the pathogen isolated from the plant tissue.

Fungal isolation

Fungi were isolated from the roots of plants growing in the field that showed symptoms of wilt and from soil infected with pathogens. The root samples were then washed under running water and the surface sterilized by dipping in a solution containing 1% sodium hypochlorite for 2 min, rinsing three times in distilled water, and drying on filter paper in a laminar airflow (Suwandi et al. 2012). They were then laid out on a Petri plate of 2% (w/v) agar and 0.1% streptomycin sulfate, and incubated for 48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012). The mycelium from the root cuttings was transferred to PDA medium using the single hyphae method. The isolate results were used for further research.

Morphological identification

The initial identification was carried out based on Leslie and Summerell (2006). The observation of cultural characteristics and the morphology of the colony included growth rates and color pigments produced on PDA media. Asexual spores and other structures were observed on the growth produced on carnation leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia and macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted to a microscope.

Pathogenicity test

This test was carried out on *A. mangium* seedlings 30 days after sowing in plastic pots containing 200 g peat soil which had previously been sterilized. Fungal isolates were grown on a potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia. This suspension was used as inoculum by pouring 1×10^6 cfu g^{-1} (colony forming unit/g) on a soil medium while the uninoculated control was sterile distilled water. Each isolate was inoculated into the soil of 10 test plants and the experiment repeated once. Disease incidence was counted as the number of diseased plants out of 10. The severity of the disease was calculated for each seedling using a score of 0 – 4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were observed—over 30 days after inoculation. The difference in disease severity and the area under the disease progress curve (AUDPC) between isolates was calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS university edition software package.

Molecular identification

Seven representative fungal isolates with distinct morphological characteristics were selected and grown in a cultivation bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). Sections of 3 – 4 day-old cultures on the PDA medium, measuring 5 mm × 5 mm, were placed on a PDB liquid medium and incubated for 3 – 4

additional days at room temperature. The fungal mycelium was harvested using vacuum filtration and frozen. DNA was extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation, Irvine, CA, USA) following manufacturer's instructions. Its concentration and quality were determined by spectrophotometry using a NanoDrop Spectrophotometry ND-1000 (NanoDrop Technologies, Montchanin, DE, U.S.A.) and stored at − 20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 (forward: 5′-ATGGGTAAGGAAGACAAGAC-3′) and EF2 (reverse: 5′-GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio-rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To determine the genetic relatedness of *F. oxysporum* from *A. mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There was a total of 577 positions in the final dataset. All positions containing gaps and missing data were eliminated. The MP tree was obtained using the subtree pruning re-grafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

Results

Disease symptoms and wilt incidences

Surveys on seedling wilt were carried out in six acacia nurseries of commercial companies; five sites in the Air Sugihan area and one in Lebong Hitam (Table 1). The incidence of disease varied between locations from 6.0% up to 36.9%. The early symptoms of seedling wilt started as the lower leaves turned yellow, then black, dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves, which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1). Observations were made by planting acacia seedlings on used soil medium from the commercial nursery, and the results showed that 56.25% of seedlings were attacked. In the commercial nurseries, this disease attacks seedlings at an average age of more than 1 month after germination and before the formation of phyllodes or modified petioles or stems.

Table 1 Disease incidence in commercial forest nurseries of *Acacia mangium* in South Sumatra

No	Location	Number of observed plants	Dead plant	Diseases incidence (%)
1	Air Sugihan A	500	34	6.8
2	Air Sugihan B	720	70	9.7
3	Air Sugihan C	720	131	18.2
4	Air Sugihan D	720	266	36.9
5	Lebong Hitam	604	36	6.0
6	Air Sugihan F	720	139	19.3

Morphological characteristics

Fifteen isolates similar to *F. oxysporum* were taken from the root tissues of diseased plants (Table 2), and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at 27 °C. The PDA cultures produced purple, pale purple and reddish-pink pigments with air hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.4 mm day^{-1}). BF06 had the slowest growth rate (7.5 mm day^{-1}) (Fig. 3). They all produced numerous microconidia on the false heads of monophialides. Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm, to 11.8 ± 4.1 µm × 3.0 ± 0.3 µm, and have zero to one septum but generally zero. The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm, to 39.7 ± 5.9 × 3.8 ± 0.4 µm, and have three to seven septa but usually three. The chlamydospores produced singly or in pairs at the terminal/intercalary have an average diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 3). Based on the morphological characteristics of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described by Leslie and Summerell (2006).

Molecular characteristics

Molecular identification by sequencing the *tef1* gene was amplified using primers EF1 and EF2 against seven selected isolates. The sequence data was determined by a comparison with those in Genbank through the BLAST and FUSARIUM-ID programs. Based on this, the sequences were confirmed as *F. oxysporum* with 84.9 – 100% similarity to Genbank and 97.3 – 99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index 0.9 and the composite index 0.8) grouped all *Fusarium* isolates within the *F. oxysporum*

Fig. 1 **a** wilt symptoms in the nursery, **b** yellowing leaves, **c** dry leaves

Table 2 Isolate origin used for pathogenicity test

Location	Host	Isolate	Number of isolates
Air Sugihan A	*A. mangium*	AF01, AF02, AF03, AF04	4
Air Sugihan B	*A. mangium*	BF05, BF06, BF07, BF08, BF09	5
Air Sugihan C	*A. mangium*	CF10	1
Air Sugihan D	*A. mangium*	DF11, DF12, DF13	3
Lebong Hitam	*A. mangium*	EF14	1
Air Sugihan F	*A. mangium*	FF15	1
Total			15

species complex. Isolates were clustered within four clades (Fig. 4). The first clade comprised BF05 and EF14, and *F. elaeidis* (MH484961.1) from *Elaeis* spp. The second clade consisted of single isolates from *A. mangium* (DF11), *F. oxysporum* (C009W and C010W) from *Cucumis melo*, and *F. triseptatum* (MH484964.1) from *Ipomoea batatas*. The third clade contained AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The fourth included DF12 and an isolate of *F. oxysporum* from *Musa* sp. *Tef1* sequences of isolates within third and fourth clade were separated from all 15 cryptic taxa of *F. oxysporum* species complex as described by Lombard et al. (2019).

Pathogenicity tests

Fusaria, isolated from diseased plant tissue, were tested for their pathogenicity in 30-day-old *A. mangium* seedlings. The results showed that symptom development began with yellowing of the lower leaves or those closest to the base of the stem, then their wilting, curving upwards, turning brown to black, drying and falling from the plant. This progresses to the top of the plant, causing it to wither and die. In addition, infected plants show symptoms of stunted growth. The first symptoms appeared 7–14 days after inoculation and were followed by total plant collapse within 1–10 days. However, some plants showed complete wilting immediately without any initial symptoms (Fig. 5). The control plants were healthy and did not show any wilting symptoms. All the isolates were able to infect plants and disease incidence and severity were significantly higher compared to the control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates caused disease severity and progress and their AUDPC values were significantly higher than other isolates and the control (Table 4). With regards to disease incidence, the *Fusarium* isolates had a high variation in percentage of disease incidence, ranging from 50 to 100%. These isolates also induced wilting severities of 1.2 – 3.2. The highest disease incidence was caused by isolate BF06 (100%), while the highest disease severity was caused by isolate DF11 (3.2). Based on disease severity, the isolates were grouped into three categories of virulence: high (score 2.0 – 4.0), moderate (score 1.2 – 2.0), and low score (0 – 1.2) virulence (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11 were included in the

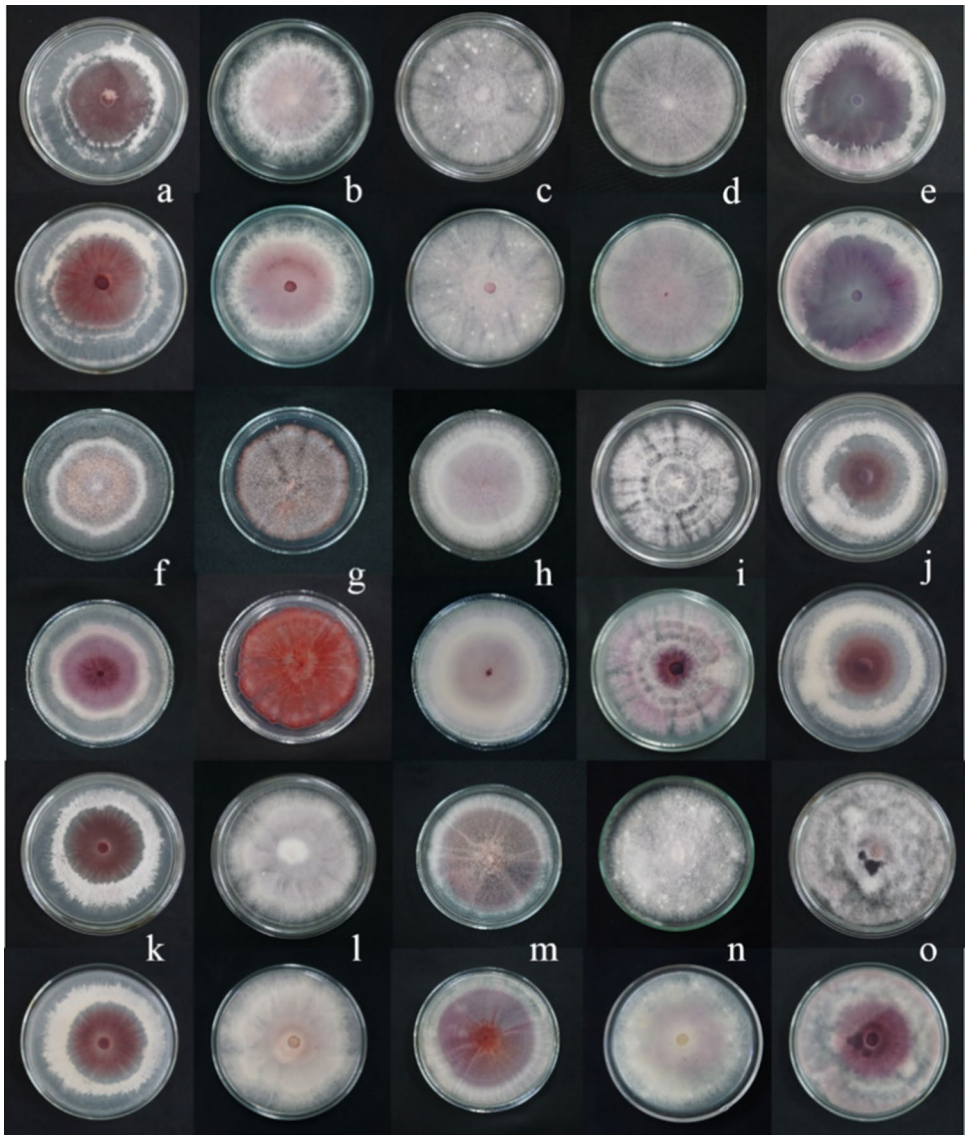


Fig. 2 Colony character on PDA; **a** AF01, **b** AF02, **c** AF03, **d** AF04, **e** BF05, **f** BF06, **g** BF07, **h** BF08, i BF09, **j** CF10, **k** DF11, **l** DF12, **m** DF13, **n** EF14, and **o** FF15. (1st, 3rd, and 5th line) Colony on top surface, (2nd, 4th, and 6th line) Colony on bottom surface

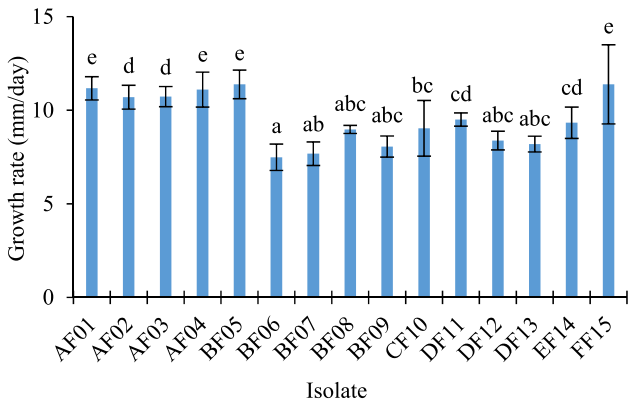


Fig. 3 Growth rate of *Fusarium oxysporum* from *Acacia mangium* on PDA medium

high virulence group (2.1 – 3.2), while FF15, BF09, BF07, AF03, and BF05 were in the moderate (1.6 – 2.0), and only EF14 had a low score. We observed that this pathogen causes vascular disease, where the pathogen could be isolated from the basal stem up to the shoots. To confirm the Koch's Postulates test, the pathogens were all re-isolated (100%) from infected plants and it was confirmed that the morphological characters of the pathogen were the same as the inoculated *Fusarium* isolates. The pathogen was not isolated from the uninoculated control plants.

Discussion

This research reports that *F. oxysporum* has been identified for the first time as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to previous

Table 3 Characteristics of the macroscopic and microscopic structure of *Fusarium oxysporum* isolated from infected plants

Isolate		Microconidia (μm)		Macroconidia (μm)		Chlamydospore (μm)	
		Characteristics	Size (L × W)	Characteristics	Size (L × W)	Characteristics	Size (D)
AF01	Top: White to dark pink Bottom: dark pink	Ellipse-allantoid, 0 – 1 septum, mostly 0 septum	10.1 ± 2.9 × 2.6 ± 0.3	3–4 septa, mostly 3	36.2 ± 5.4 × 4.1 ± 0.4	Terminal/intercalary, single/pair	8.1 ± 1.0
AF02	Top: White to pale violet Bottom: Pale violet	Oval -reniform, 0 septum	6.8 ± 1.0 × 2.6 ± 0.3	3–4 septa, mostly 3	38.1 ± 5.3 × 3.9 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.9
AF03	Top: white Bottom: pale violet	Ellipse-oval shaped, 0 septum	6.5 ± 1.0 × 2.7 ± 0.3	3–4 septa, mostly 3	39.7 ± 5.9 × 3.8 ± 0.4	Terminal/intercalary, single/pair	7.2 ± 1.1
AF04	Top: white Bottom: Pale violet	Ellipse-reniform shaped, 0 septum	6.7 ± 1.4 × 2.6 ± 0.4	3–4 septa, mostly 3	38.3 ± 5.8 × 3.8 ± 0.3	Terminal/intercalary, single/pair	7.7 ± 1.0
BF05	Top: White to pale violet Bottom: Violet	Ellipse-allantoid, 0 – 1 septum, mostly 0	8.6 ± 2.5 × 2.9 ± 0.4	3–4 septa, mostly 3	33.6 ± 5.4 × 3.9 ± 0.5	Terminal/intercalary, single/pair	7.3 ± 0.9
BF06	Top: White to pale violet Bottom: Violet	Allantoid shaped, 0 – 2 septa, mostly 0 septum	7.8 ± 2.3 × 2.8 ± 0.5	3–4 septa, mostly 3	37.8 ± 6.9 × 4.1 ± 0.4	Terminal/intercalary, single/pair	9.5 ± 0.8
BF07	Top: White to dark pink Bottom: Dark pink	Oval-allantoid shaped, 0 – 2 septa, mostly 0—1septum	7.6 ± 1.8 × 3.5 ± 0.3	3–4 septa, mostly 3	37.5 ± 5.3 × 3.7 ± 0.4	Terminal/intercalary, single/pair	7.3 ± 0.6
BF08	Top: White to pale violet Bottom: Pale violet	Allantoid shaped, 0 – 2 septa, mostly 0 septum	9.2 ± 2.6 × 2.6 ± 0.3	3–4 septa, mostly 3	36.7 ± 5.1 × 4.0 ± 0.6	Terminal/intercalary, single/pair	7.0 ± 1.0
BF09	Top: White Bottom: Violet to pale violet	Oval-ellipse shaped, 0 septum	7.6 ± 1.8 × 2.9 ± 0.4	3–4 septa, mostly 3	37.4 ± 6.6 × 4.0 ± 0.3	Terminal/intercalary, single/pair	6.4 ± 0.6
CF10	Top: White to dark pink Bottom: dark pink	Ellipse-allantoid, 0 – 1 septum, mostly 0 septum	11.8 ± 4.1 × 3.0 ± 0.3	3–7 septa, mostly 3	39.2 ± 6.2 × 4.1 ± 0.3	Terminal/intercalary, single/pair	9.0 ± 1.4
DF11	Top: White to dark pink Bottom: dark pink	Ellipse-allantoid, 0 – 1 septum, mostly 0 septum	8.0 ± 2.2 × 2.4 ± 0.3	3–4 septa, mostly 3	36.5 ± 4.5 × 3.9 ± 0.3	Terminal/intercalary, single/pair	7.2 ± 0.7
DF12	Top: white to pale violet Bottom: pale violet	Ellipse-allantoid shaped, 0 – 1 septum, mostly 0 septum	7.4 ± 1.3 × 2.4 ± 0.3	3–4 septa, mostly 3	37.8 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	10.0 ± 2.5
DF13	Top: white to pale violet Bottom: pale violet	Ellipse-allantoid, 0 – 1 septum, mostly 0 septum	10. 3 ± 2.7 × 2.5 ± 0.3	3–4 septa, mostly 3	38.8 ± 5.9 × 3.6 ± 0.3	Terminal/intercalary, single/pair	6.6 ± 0.7
EF14	Top: white Bottom: pale violet	Oval shaped, 0 septum	5.5 ± 1.0 × 2.8 ± 0.5	3–4 septa, mostly 3	36.9 ± 4.8 × 3.8 ± 0.4	Terminal/intercalary, single/pair	6.8 ± 0.8
FF15	Top: White Bottom: Violet	Oval-ellipse, 0 – 1 septum, mostly 0 septum	8.0 ± 1.7 × 2.7 ± 0.3	3–4 septa, mostly 3	37.0 ± 5.4 × 4.0 ± 0.4	Terminal/intercalary, single/pair	7.9 ± 1.6
Mean			8.1 ± 1.7 × 2.8 ± 0.3		37.5 ± 5.6 × 3.9 ± 0.4		7.7 ± 1.0

studies, *F. oxysporum* was reported as the cause of seedling wilt in *A. koa* in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen was reported to attack germinated acacia seeds, resulting in damping-off, and the identification of this was made solely on morphological characters (Widyastuti et al. 2013). Our

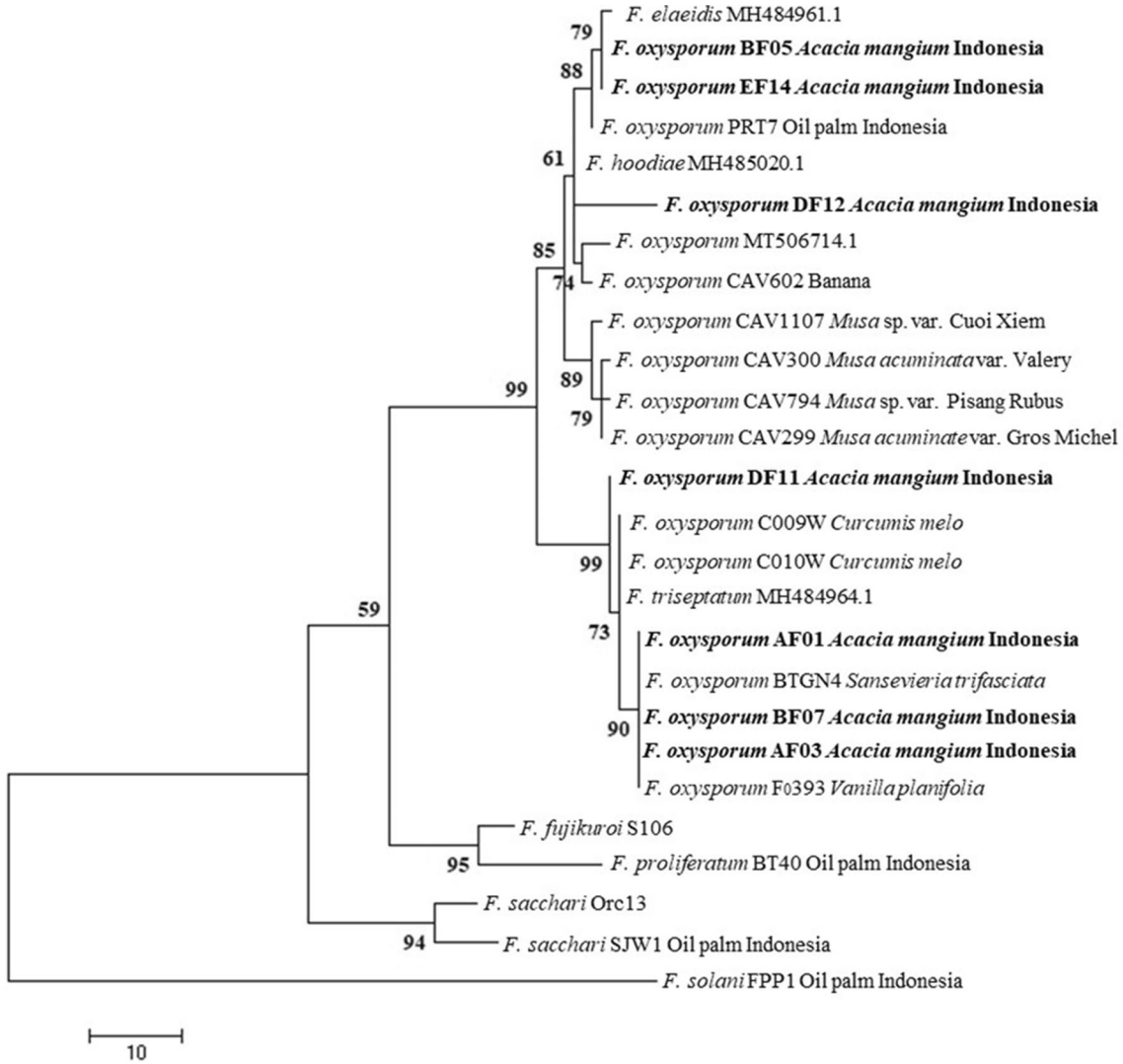


Fig. 4 One out of the nine most parsimonious trees showing the genetic relatedness, represented by the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium*-(in bold), closely related *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host species and countries of origin are given with the representative isolates. A bootstrap test with 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The genetic distance is indicated by the scale bar

research confirms that *F. oxysporum*, identified by morphological and molecular methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana* Lamb.

The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2, respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in India with diseases severity of 16.9% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85.0% severity (Gardner 1980), and damping-off on *A. mangium* in Papua, Indonesia with unknown disease severity (Widyastuti et al. 2013).

The results here have also shown that *F. oxysporum* is a pathogen causing vascular wilt in *A. mangium* seedlings, which is evident in the isolation in the shoots. The pathogens infect the roots, then enter and multiply along the xylem vessels, and are translocated to the shoots through water movement. Browning in the xylem tissue, which is typical of vascular wilt disease (Meena and Roy 2020), was also exhibited in the inoculated plants.

F. oxysporum was determined based on morphological identification, both visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and

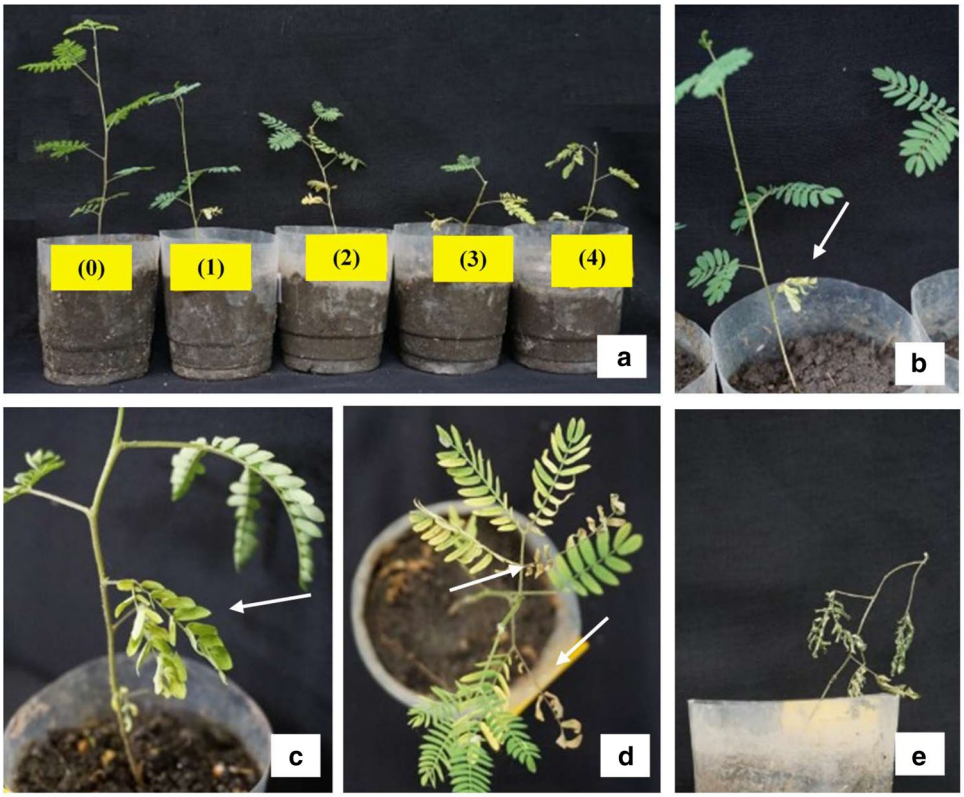


Fig. 5 Disease severity rate: **a** from left: healthy plant to 100% wilted leaves (scale 0 – 4); **b** initial symptoms: from lowest leaf, yellowing leaves; **c**, **d** advanced symptoms: curved leaves, dry leaves, falling leaves; **e** dead plant

Table 4 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings inoculated with *F. oxysporum*

Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3^{ab}	31.0^{ab}
AF02	80	2.4^{a}	46.4^{a}
AF03	80	1.9^{ab}	32.6^{ab}
AF04	80	2.5^{a}	33.9^{ab}
BF05	50	2.0^{ab}	45.5^{ab}
BF06	100	3.1^{a}	59.5^{a}
BF07	60	1.9^{ab}	32.6^{ab}
BF08	70	2.1^{ab}	37.5^{ab}
BF09	70	1.6^{ab}	23.6^{ab}
CF10	90	2.7^{a}	37.9^{a}
DF11	90	3.2^{a}	55.7^{a}
DF12	80	2.8^{a}	49.8^{a}
DF13	80	2.9^{a}	54.7^{a}
EF14	60	1.2^{ab}	15.3^{ab}
FF15	50	1.6^{ab}	32.0^{ab}
Control	0	0^{b}	0.0^{b}
P-Value		0.0013	0.0007

The numbers followed by the same letter in the column are not significantly different in the LSD test at 5%

Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the most similar to the *F. oxysporum* species complex from *Elaeis* sp. (*F. elaeidis*) (Lombard et al. 2019), *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar and Mohd 2019).

Field observation of the disease in the commercial nursery showed that about 36.9% of plants died through *Fusarium* wilt. This attack caused large economic losses because these nurseries provide seedlings for commercial gardening in South Sumatra over 1,324,653 hectares. The high disease incidence in the field was possibly caused by the previous use of seedlings infected with the pathogens, which led to rapid development and accumulation through the nursery cycle. This is evident in the high disease incidence on soil media. It is likely that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt comes from infected soil and that pathogens can survive a long periods in the soil, and in other plant debris (Postic et al. 2012; Altinok 2013).

Conclusions

This study is the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt in South Sumatra, Indonesia. The pathogen was confirmed through morphological and *tef1* gene sequencing and Koch's postulate. The main source of a primary inoculum which causes *Fusarium* wilt disease in South Sumatra comes from seedling soils contaminated with pathogens.

References

Altinok HH (2013) Fusarium species isolated from common weeds in eggplant fields and symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161:335–340

Altinok HH, Can C, Altinok MA (2018) Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from Turkey with ISSR markers and DNA sequence analyses. Eur J Plant Pathol 150:609–621

Bakar MA, Mohd M (2019) Characterization of *Fusarium oxysporum* isolated from agricultural and non-agricultural ecosystems in Malaysia. Unpublished

Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S (2011) Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol 28(4):166–172

Dobbs JT, Kim MS, Dudley NS, Klopfenstein NB, Yeh A, Hauff RD, Stewart JE (2020) Whole genome analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) and the development of molecular tools for early detection and monitoring. BMC Genom 21:1–15

Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris*. Arch Phytopathol Pflanzenschutz 43(2):174–190

Edel-Hermann V, Lecomte C (2019) Current status of *Fusarium oxysporum* formae speciales and races. J Phytopathol 109:512–530

Gardner DE (1980) Acacia koa seedling wilt caused by *Fusarium oxysporum* f. sp. koae, f. sp. nov. J Phytopathol 70(7):594–597

Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev Phytopathol 55:23–39

Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd. A fast growing tree for tropical plantations. J For Sci 29(1): 1–14.

Jiménez-Díaz RM, Castillo P, MdelM J-G, Landa BB, Navas-Cortés JA (2015) Fusarium wilt of chickpeas: biology, ecology and management. Crop Prot 73:16–27

Kapoor S, Harsh NSK, Sharma SK (2004) A new wilt disease of *Acacia nilotica* caused by *Fusarium oxysporum*. J Trop for Sci 16(4):453–462

Kee YJ, Zakaria L, Mohd MH (2020) Morphology, phylogeny and pathogenicity of Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant Pathol 69(3):442–454

Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin S, Gautheron N, Grisoni M (2016) *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla. Plant Pathol 65(4):612–625

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874

Leslie JF, Summerell BA (2006) The fusarium laboratory manual. Blackwell Publishing, Oxford

Lombard L, Lamprecht SC, Crous PW (2019) Epitypification of *Fusarium oxysporum*——clearing the taxonomic chaos. Persoonia 43:1–15

Luo X, Yu C (2020) First report of damping-off disease caused by *Fusarium oxysporum* in *Pinus massoniana* in China. J Plant Dis Prot 127:401–409

Meena RP, Roy S (2020) Morphological and molecular characterization of *Fusarium* sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. J Appl Res Med Aromat Plants 100244

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York

O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Sci USA 95:2044–2049

Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on *Fusarium* wilt, focusing on bananas. Appl Soil Ecol 132:20–33

Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium species isolated from weeds and plant debris in Croatia. J Phytopathol 160:76–81

Statistics Indonesia (2018) Timber culture establishment. BPS-Statistics Indonesia, Jakarta

Sun FF, Sun SL, Zhu L, Duan CX, Zhu ZD (2019) Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Prot 117:77–85

Suwandi AS, Kondo N (2012) Common spear rot of oil palm in Indonesia. Plant Dis 96(4):537–543

Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant Pathol 40(2):238–247

Widyastuti SM, Tasik S, Harjono, (2013) Infection process of *Fusarium oxysporum* fungus: a cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2):110c118