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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.
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1 # The Identification and Pathogenicity of *Fusarium oxysporum* causing
2 # Acacia Seedling Wilt Disease

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13 **Abstract**

14 *Fusarium oxysporum* is an important pathogen in nurseries of commercial acacia in
15 South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of
16 chlorosis in the lower leaves and develops into the shoots; consequently, the plants
17 wither and die. This research aims to identify the pathogenic species causing seedling
18 wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F.*
19 *oxysporum* with varying colony size and color pigment were confirmed by observing
20 the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The
21 pathogenicity test showed that all isolates could infect plants with wilt severity reaching
22 80%, and the *Fusarium pathogen* was verified as causing vascular disease.
23 Furthermore, Koch's postulate was confirmed by re-isolating the *F.oxysporum* isolate.
24 The primary source of pathogenic inoculums in commercial nurseries at South Sumatra
25 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).

51 Previous studies have reported seedling wilt disease affecting *Acacia koae* in
52 Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F.*
53 *oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House
54 of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F.*
55 *oxysporum*, has been found to cause damping-off disease in seedlings six days after
56 germination (Widyastuti et al. 2013). This research aims to identify the pathogenic
57 species causing wilt disease in advanced stage seedlings of *A. mangium* and the
58 disease's pathogenicity.
59
60 **MATERIAL AND METHODS**
61 **Survey and sampling**
62 Soil and diseased plant samples were collected from five commercial company-
63 owned acacia nursery estates with a seedling wilt problem. The diseased plants showed
64 initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up.
65 The symptoms started from the lower leaves, moving on to the upper leaves and the
66 shoots. Plant samples showing wilting symptoms were collected and stored in a cool
67 box during the process. To determine soil infectivity, acacia seeds were sown using a
68 seedbed, and to accelerate germination, they were soaked in hot water (± 95 $^{\circ}$C) and left
69 to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the
70 seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three
71 times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate
72 the radicula germination. The germinated seeds were planted on infested field nursery
73 medium. The infected seedlings from the field and the infected soil nursery medium
74 were taken and the pathogen was isolated from the plant tissue.
75

76 **Fungal isolation**

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

87

88 **Morphological identification**

89 The initial identification was carried out based on the Leslie and Summerell
90 (2006) method. The observation of cultural characteristics and the morphology of the
91 colony included growth rates and color pigments produced on PDA media. Asexual
92 spores and other structures were observed based on the growth produced on carnation
93 leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX
94 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta,
95 Indonesia). Measurements were carried out on 100 spores of microconidia,
96 macroconidia, and chlamydospores using Image Raster 3.0 software with magnification
97 adjusted to a microscope.

98

99 **Pathogenicity test**

100 A pathogenicity test was carried out on *A. mangium* seedlings 30 days after
101 sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which
102 had previously been sterilized using an autoclave. Fungal isolates were grown in a
103 potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures
104 were incubated for three days using a shaker at a speed of 120 rpm to produce large
105 quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a
106 soil medium, while the uninoculated control was watered only with sterile distilled
107 water. Each isolate was inoculated on 10 test plants and the experiment was repeated
108 once. Disease incidence was counted by the number of diseased plants out of the 10
109 tested plants. The severity of the disease was calculated using a score of 0‒4, where 0
110 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted,
111 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1‒30 days
112 after inoculation. The difference in disease severity and the area under the disease
113 progress curve (AUDPC) between isolates was calculated through ANOVA and
114 Tukey's HSD test. An analysis was performed using the SAS university edition
115 software package.
116
117 **Molecular identification**
118 Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle
119 containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3‒
120 4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB
121 liquid medium and incubated for 3‒4 days at room temperature. Meanwhile, the fungal
122 mycelium was harvested using vacuum filtration and then it was frozen. The DNA was
123 extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation,
124 California, USA) following manufacturer's instructions. Its concentration and quality

125 were determined by spectrophotometry using a NanoDrop Spectrophotometry ND‑
126 1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at ‑20 °C
127 until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1
128 (forward: 5′ ‑ATGGGTAAGGAAGACAAGAC ‑3′) and EF2 (forward:
129 5′‑GGAAGTACCAGTGATCATGTT‑3′) (O'Donnell et al. 1998). PCR was carried out
130 in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany)
131 (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each
132 primer, and 2 µL of DNA template. The amplification was performed using a PCR
133 Cycler Termal C1000 Touch ™ (Bio‑ rad, USA). The initial denaturation was
134 performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40
135 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65
136 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE,
137 Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by
138 comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID
139 databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia*
140 *mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using
141 Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There
142 was a total of 583 positions in the final dataset. All positions containing gaps and
143 missing data were eliminated. The MP tree was obtained using the subtree pruning
144 regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).
145
146 **RESULTS AND DISCUSSION**
147 **Results**
148 ***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 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.

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).

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

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

275

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282 **REFERENCES**

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

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

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

291 Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S
292 (2011) Isolation and characterization of two strains of *Fusarium oxysporum*
293 causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol
294 28(4): 166–172.

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

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

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

303 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 Click here to access/download;Table;Table.docx

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*.

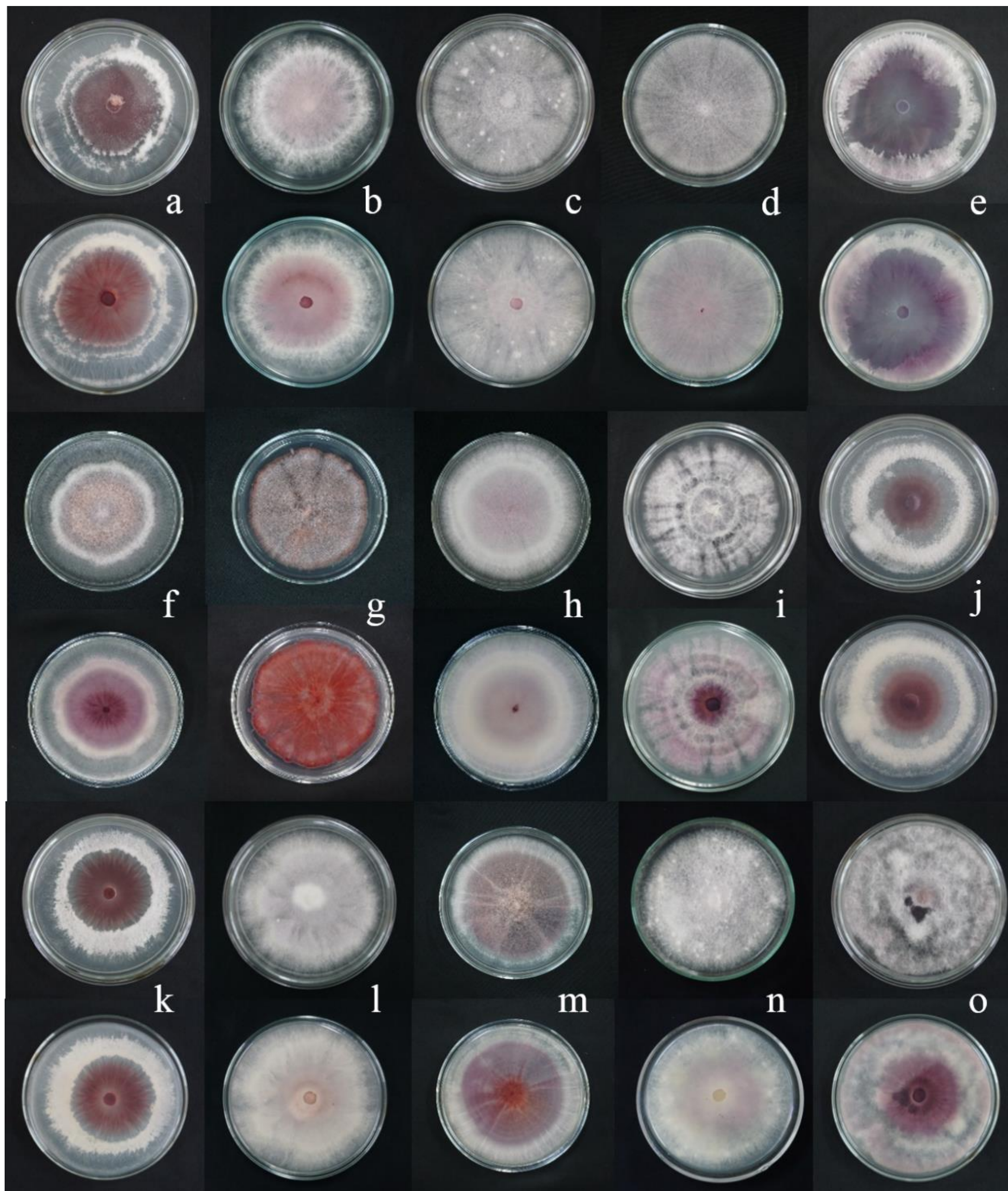
Isolate	Disease incidence (%)	Disease severity	AUDPC
AF01	80	2.3 ab	31.0 ab
AF02	80	2.4 a	46.4 a
AF03	80	1.9 ab	32.6 ab
AF04	80	2.5 a	33.9 ab
BF05	50	2.0 ab	45.5 ab
BF06	100	3.1 a	59.5 a
BF07	60	1.9 ab	32.6 ab
BF08	70	2.1 ab	37.5 ab
BF09	70	1.6 ab	23.6 ab
CF10	90	2.7 a	37.9 a
DF11	90	3.2 a	55.7 a
DF12	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

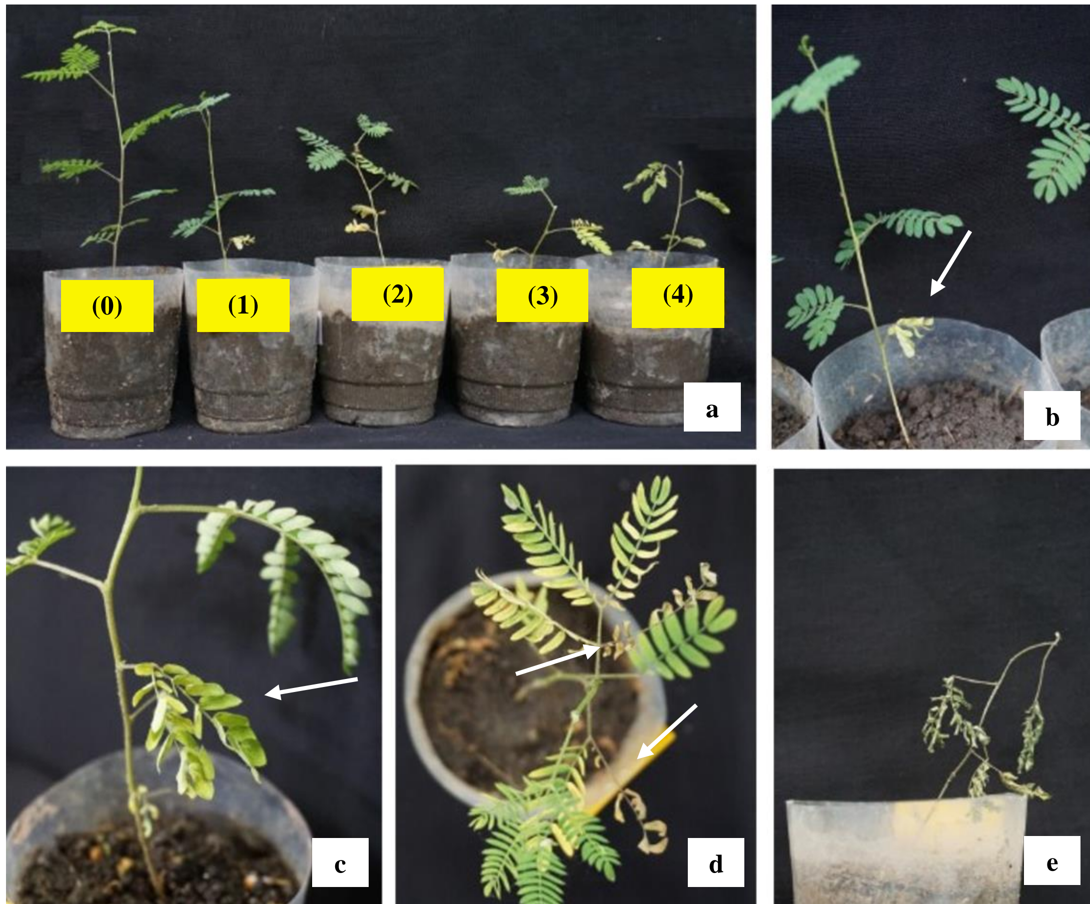
369 Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f),

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

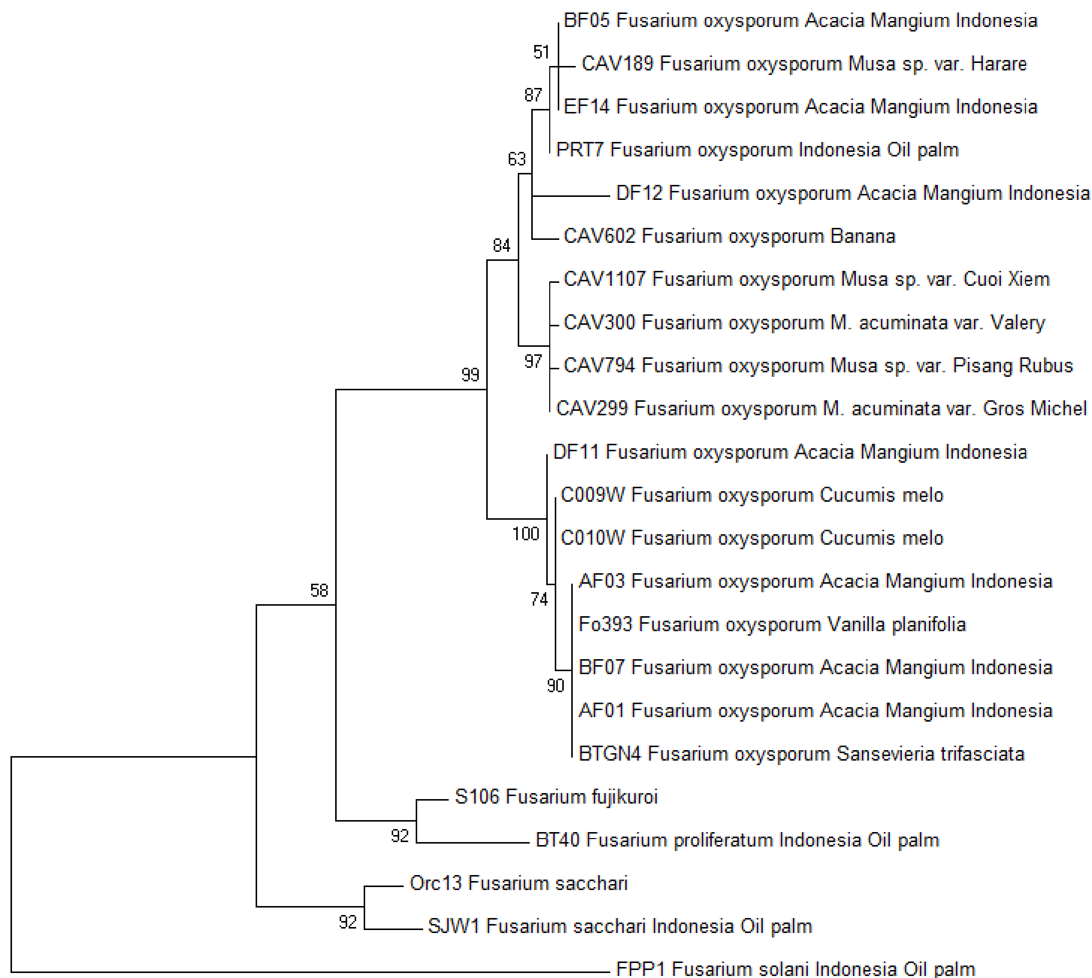
373

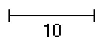


374 Fig. 3 Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial

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.

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Thank you very much for your consideration of the manuscript.

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REVIEW PROCESS

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Your Submission

1 message

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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.

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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').

____

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REVIEWER 1

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:

See next page

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.

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

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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.

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

27

28 INTRODUCTION

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

41 *F. oxysporum* is a soil-borne pathogen with a very wide host range and is
42 common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann
43 and Lecomte 2019) including forest and industrial ~~plants~~ (Widyastuti et al. 2013). This
44 pathogen attacks all phases of growth (vegetative and generative) and is able to survive
45 on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes
46 vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The
47 symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's
48 vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al.
49 2019). This species is a dangerous pathogen for plants cultivated both on open land and
50 in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

51 Previous studies have reported seedling wilt disease affecting *Acacia koae* in
52 Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F.*
53 *oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House
54 of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F.*
55 *oxysporum*, has been found to cause damping-off disease in seedlings six days after
56 germination (Widyastuti et al. 2013). This research aims to identify the pathogenic
57 species causing wilt disease in advanced stage seedlings of *A. mangium* and the
58 disease's pathogenicity.
59

60 **MATERIAL AND METHODS**

61 **Survey and sampling**

62 Soil and diseased plant samples were collected from five commercial company-
63 owned acacia nursery estates with a seedling wilt problem. The diseased plants showed
64 initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up.
65 The symptoms started from the lower leaves, moving on to the upper leaves and the
66 shoots. Plant samples showing wilting symptoms were collected and stored in a cool
67 box during the process. To determine soil infectivity, acacia seeds were sown using a
68 seedbed, and to accelerate germination, they were soaked in hot water (± 95 °C) and left
69 to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the
70 seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three
71 times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate
72 the radicula germination. The germinated seeds were planted on infested field nursery
73 medium. The infected seedlings from the field and the infected soil nursery medium
74 were taken and the pathogen was isolated from the plant tissue.
75

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 x 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

100 A pathogenicity test was carried out on *A. mangium* seedlings 30 days after
101 sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which
102 had previously been sterilized using an autoclave. Fungal isolates were grown in a
103 potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures
104 were incubated for three days using a shaker at a speed of 120 rpm to produce large
105 quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a
106 soil medium, while the uninoculated control was watered only with sterile distilled
107 water. Each isolate was inoculated on 10 test plants and the experiment was repeated
108 once. Disease incidence was counted by the number of diseased plants out of the 10
109 tested plants. The severity of the disease was calculated using a score of 0–4, where 0
110 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted,
111 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1–30 days
112 after inoculation. The difference in disease severity and the area under the disease
113 progress curve (AUDPC) between isolates was calculated through ANOVA and
114 Tukey's HSD test. An analysis was performed using the SAS university edition
115 software package.
116

117 **Molecular identification**

118 Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle
119 containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3–
120 4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB
121 liquid medium and incubated for 3–4 days at room temperature. Meanwhile, the fungal
122 mycelium was harvested using vacuum filtration and then it was frozen. The DNA was
123 extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation,
124 California, USA) following manufacturer's instructions. Its concentration and quality

125 were determined by spectrophotometry using a NanoDrop Spectrophotometry ND - 126 1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at -20 °C 127 until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1 128 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (forward: 129 5′-GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out 130 in 50 μL of the reaction mixture containing 20 μL Master Mix (Eppendorf, Germany) 131 (1.25 GoTaq DNA polymerase, 0.2 μM of each dNTP, 2 × PCR buffer), 1 μL of each 132 primer, and 2 μL of DNA template. The amplification was performed using a PCR 133 Cycler Termal C1000 Touch ™ (Bio- rad, USA). The initial denaturation was 134 performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40 135 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65 136 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE, 137 Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by 138 comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID 139 databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia* 140 *mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using 141 Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There 142 was a total of 583 positions in the final dataset. All positions containing gaps and 143 missing data were eliminated. The MP tree was obtained using the subtree pruning 144 regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).

145

146 RESULTS AND DISCUSSION

147 Results

148 *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.

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).

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

272 through morphological and *tef1* gene sequencing and Koch's postulate. The main
273 source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra
274 comes from a soil seedling medium infested with pathogens.
275
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282 **REFERENCES**

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

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

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

291 Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S
292 (2011) Isolation and characterization of two strains of *Fusarium oxysporum*
293 causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol
294 28(4): 166–172.

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

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

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

303 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.

Click here to access/download;Table;Table.docx

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%.

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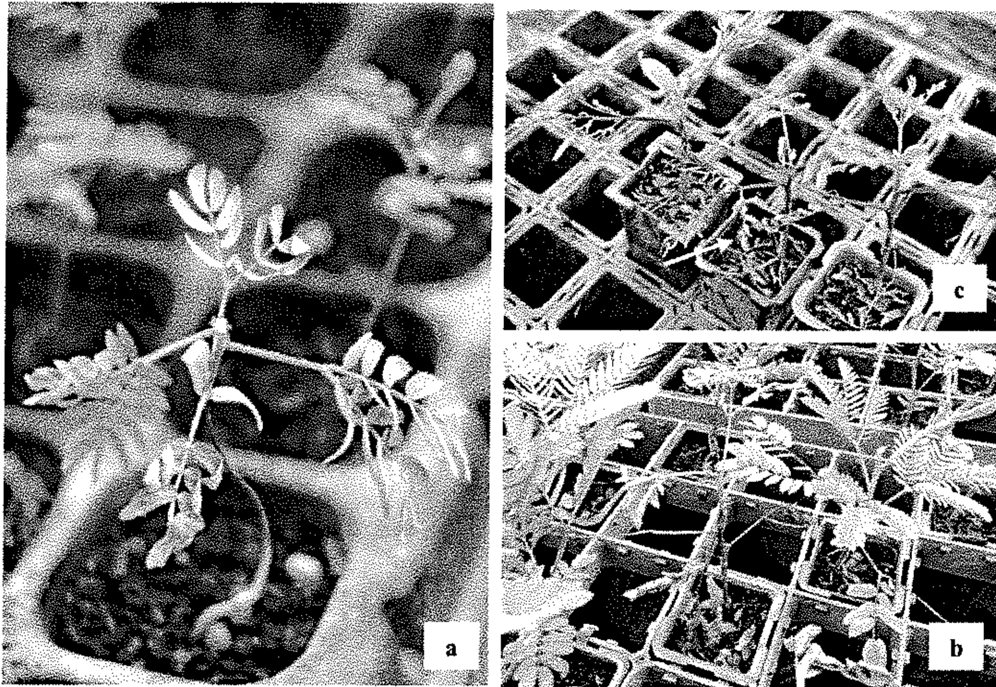
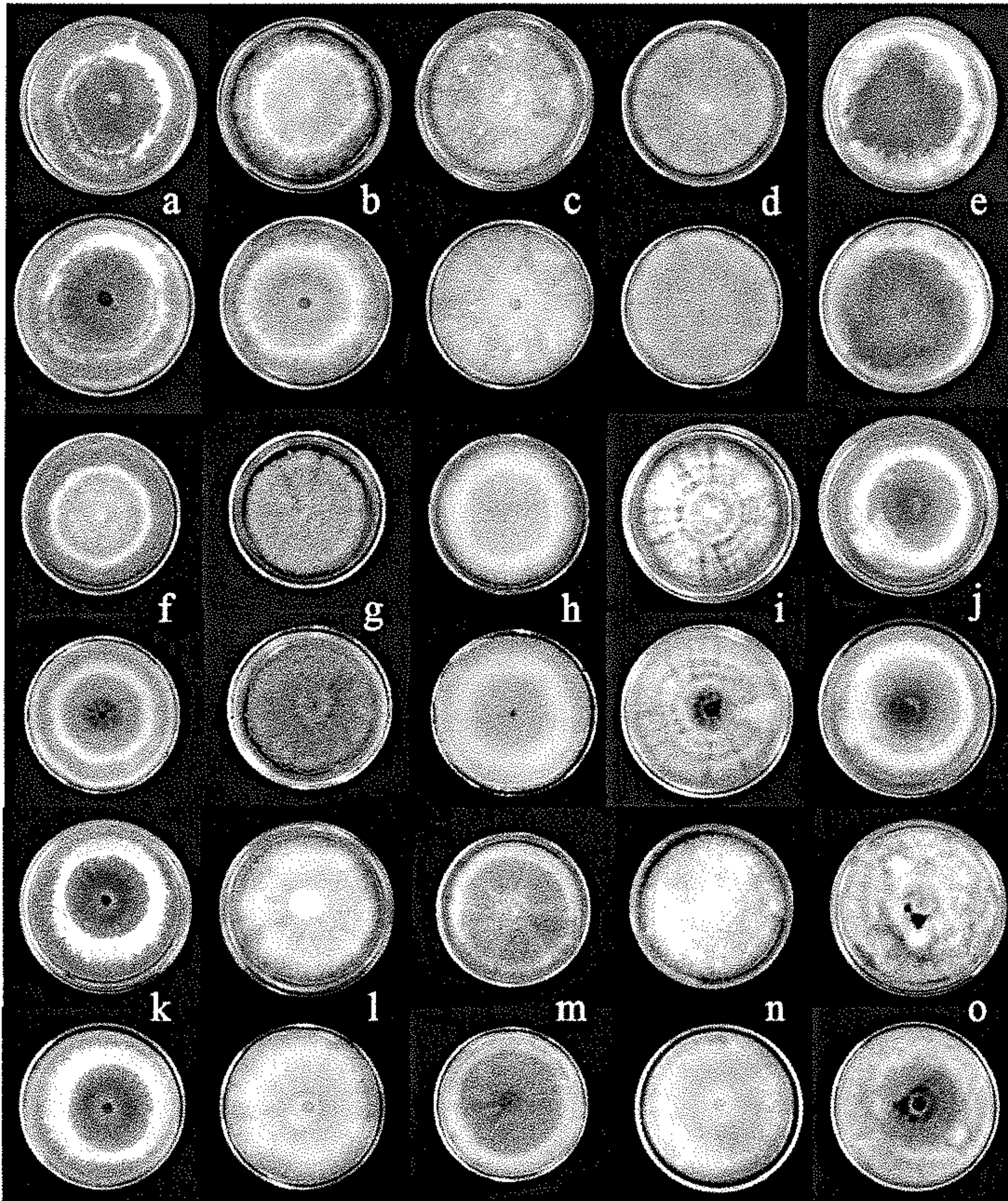
366



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



368

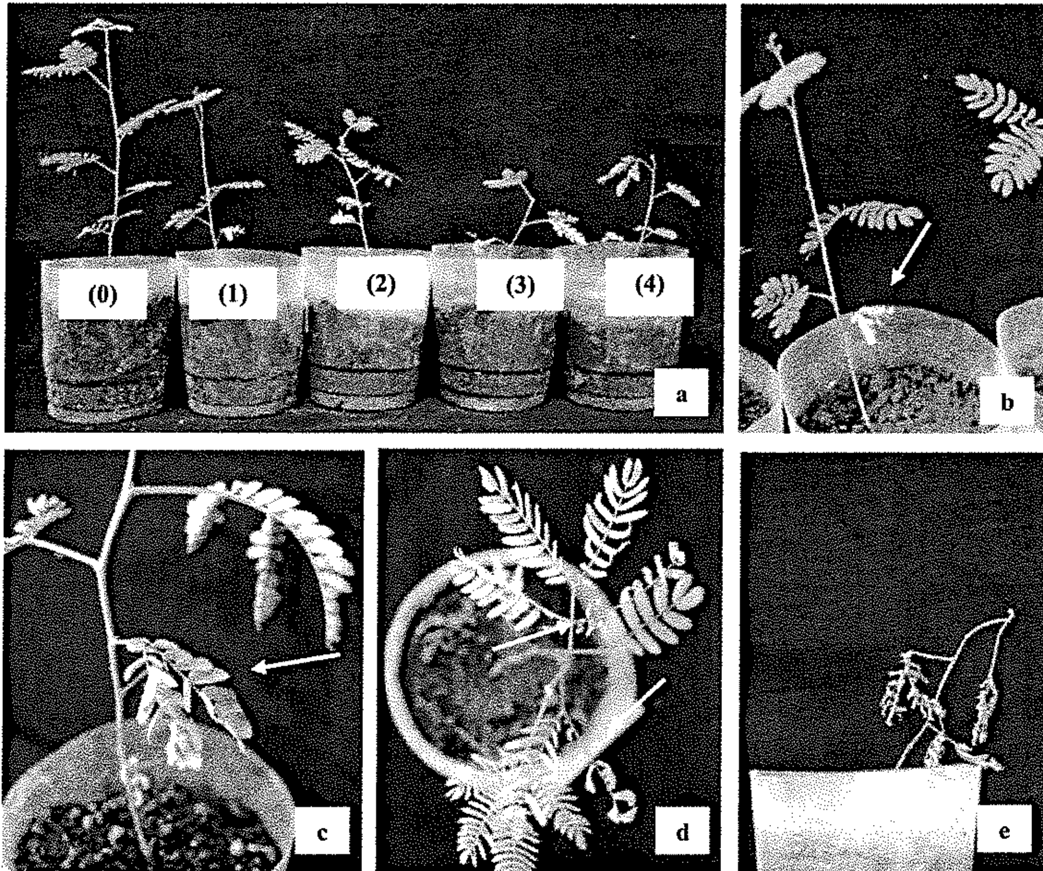
369 Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f),

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

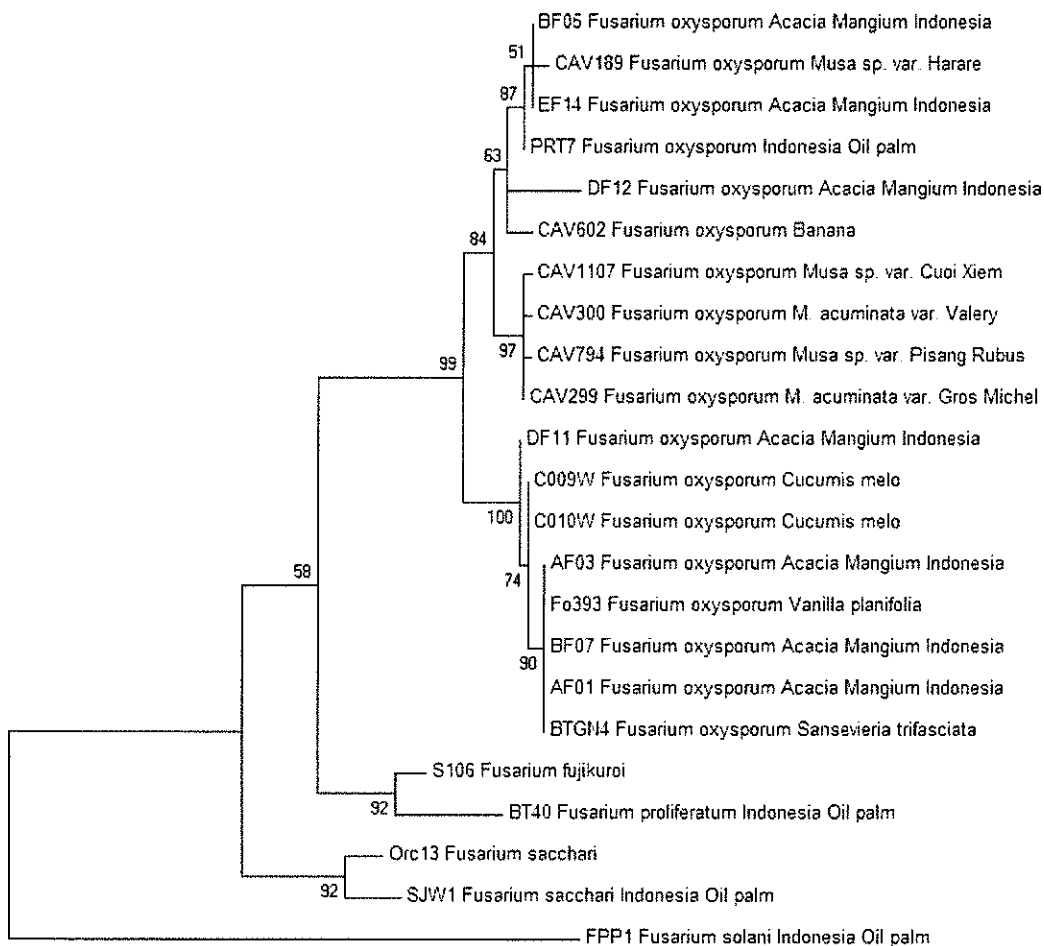
373



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.

REVIEWER 2

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
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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.

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

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13 **Abstract**
14 *Fusarium oxysporum* is an important pathogen in nurseries of commercial acacia in
15 South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of
16 chlorosis in the lower leaves and develops into the shoots; consequently, the plants
17 wither and die. This research aims to identify the pathogenic species causing seedling
18 wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F.*
19 *oxysporum* with varying colony size and color pigment were confirmed by observing
20 the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The
21 pathogenicity test showed that all isolates could infect plants with wilt severity reaching
22 80%, and the *Fusarium pathogen* was verified as causing vascular disease.
23 Furthermore, Koch's postulate was confirmed by re-isolating the *F.oxysporum* isolate.
24 The primary source of pathogenic inoculums in commercial nurseries at South Sumatra
25 comes from soil seedling media infested with pathogens.

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

27

28 INTRODUCTION

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

41 *F. oxysporum* is a soil-borne pathogen with a very wide host range and is
42 common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann
43 and Lecomte 2019) including forest and industrial plants (Widyastuti et al. 2013). This
44 pathogen attacks all phases of growth (vegetative and generative) and is able to survive
45 on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes
46 vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The
47 symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's
48 vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al.
49 2019). This species is a dangerous pathogen for plants cultivated both on open land and
50 in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

51 Previous studies have reported seedling wilt disease affecting *Acacia koae* in
52 Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F.*
53 *oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House
54 of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F.*
55 *oxysporum*, has been found to cause damping-off disease in seedlings six days after
56 germination (Widyastuti et al. 2013). This research aims to identify the pathogenic
57 species causing wilt disease in advanced stage seedlings of *A. mangium* and the
58 disease's pathogenicity.
59
60 **MATERIAL AND METHODS**
61 **Survey and sampling**
62 Soil and diseased plant samples were collected from five commercial company-
63 owned acacia nursery estates with a seedling wilt problem. The diseased plants showed
64 initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up.
65 The symptoms started from the lower leaves, moving on to the upper leaves and the
66 shoots. Plant samples showing wilting symptoms were collected and stored in a cool
67 box during the process. To determine soil infectivity, acacia seeds were sown using a
68 seedbed, and to accelerate germination, they were soaked in hot water (± 95 ˚C) and left
69 to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the
70 seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three
71 times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate
72 the radicula germination. The germinated seeds were planted on infested field nursery
73 medium. The infected seedlings from the field and the infected soil nursery medium
74 were taken and the pathogen was isolated from the plant tissue.
75

Commented [USER1]: How to sample?

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

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

76 **Fungal isolation**

77 Fungi were isolated from the roots of plants showing the symptoms of seedling
78 wilt, both from the field and the soil infected with pathogens. Then, the root samples
79 were washed under running water, and the surface was sterilized by dipping them in a
80 solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using
81 sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et
82 al. 2012). Then, they were planted in a Petri plate containing agar water with 2%(w/v)
83 agar and 0.1% Streptomycin-sulfate and incubated for 2 × 24 h (Gardner 1980; Leslie
84 and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root
85 cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium
86 using the single hyphae method. The isolate results were used for further research.
87
88 **Morphological identification**

89 The initial identification was carried out based on the Leslie and Summerell
90 (2006) method. The observation of cultural characteristics and the morphology of the
91 colony included growth rates and color pigments produced on PDA media. Asexual
92 spores and other structures were observed based on the growth produced on carnation
93 leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX
94 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta,
95 Indonesia). Measurements were carried out on 100 spores of microconidia,
96 macroconidia, and chlamydospores using Image Raster 3.0 software with magnification
97 adjusted to a microscope.
98
99 **Pathogenicity test**

100 A pathogenicity test was carried out on *A. mangium* seedlings 30 days after
101 sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which
102 had previously been sterilized using an autoclave. Fungal isolates were grown in a
103 potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures
104 were incubated for three days using a shaker at a speed of 120 rpm to produce large
105 quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a
106 soil medium, while the uninoculated control was watered only with sterile distilled
107 water. Each isolate was inoculated on 10 test plants and the experiment was repeated
108 once. Disease incidence was counted by the number of diseased plants out of the 10
109 tested plants. The severity of the disease was calculated using a score of 0–4, where 0
110 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted,
111 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1−30 days
112 after inoculation. The difference in disease severity and the area under the disease
113 progress curve (AUDPC) between isolates was calculated through ANOVA and
114 Tukey's HSD test. An analysis was performed using the SAS university edition
115 115 software package.
116 116

117 **Molecular identification**

118 Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle
119 containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3
120 4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB
121 liquid medium and incubated for 3–4 days at room temperature. Meanwhile, the fungal
122 mycelium was harvested using vacuum filtration and then it was frozen. The DNA was
123 extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation,
124 California, USA) following manufacturer's instructions. Its concentration and quality

125 were determined by spectrophotometry using a NanoDrop Spectrophotometry ND-
126 1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at -20 °C
127 until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1
128 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (forward:
129 5′ GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out
130 in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany)
131 (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each
132 primer, and 2 µL of DNA template. The amplification was performed using a PCR
133 Cycler Termal C1000 Touch ™ (Bio-rad, USA). The initial denaturation was
134 performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40
135 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65
136 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE,
137 Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by
138 comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID
139 databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia*
140 *mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using
141 Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There
142 was a total of 583 positions in the final dataset. All positions containing gaps and
143 missing data were eliminated. The MP tree was obtained using the subtree pruning
144 144 regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).
145 145

146 **RESULTS AND DISCUSSION**

147 **Results**

148 ***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.

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).

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
267267 pathogens can also survive in other plant debris (Postic et al. 2012; Altinok 2013)
268268

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.

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282 **REFERENCES**

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

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

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

291 Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S
292 (2011) Isolation and characterization of two strains of *Fusarium oxysporum*
293 causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol
294 28(4): 166–172.

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

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

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

303 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* sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its management strategies. Journal of Applied Research on Medicinal and Aromatic Plants 100244.

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

332 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.

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

337 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.

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 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 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-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.

Table Click here to access/download;Table;Table.docx

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

353 353

354 3 Table 2 Isolate origin used for pathogenicity test.
5
4

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

361

Mean

8.1 ± 1.7 × 2.8

±
0
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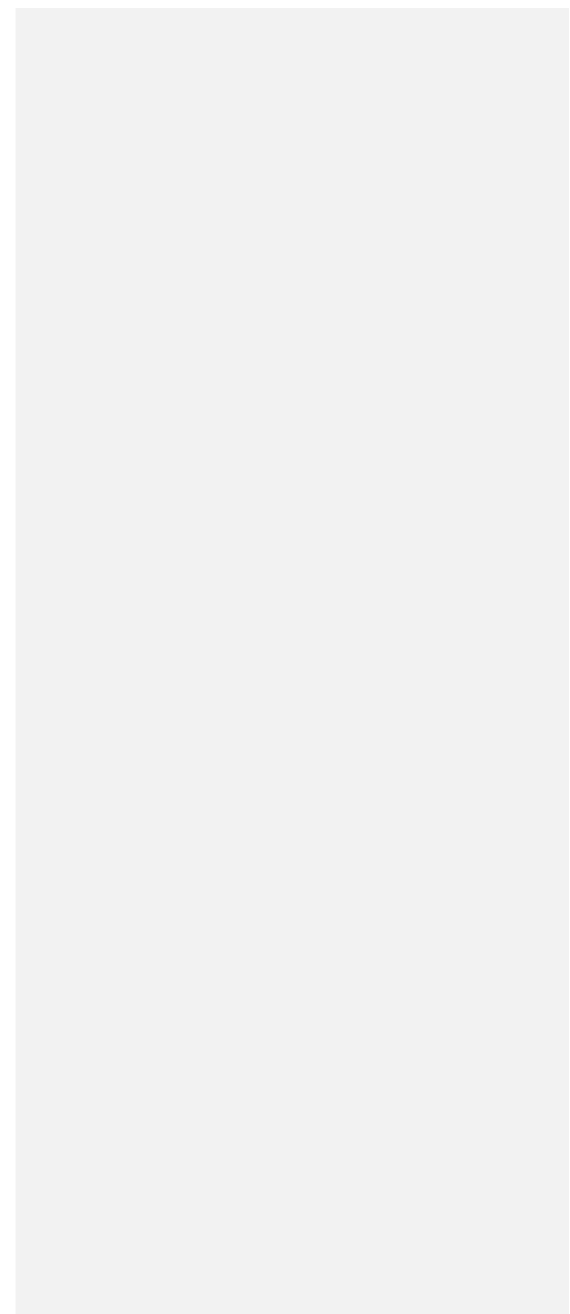
3

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 Click here to access/download;Figure;Figure.docx

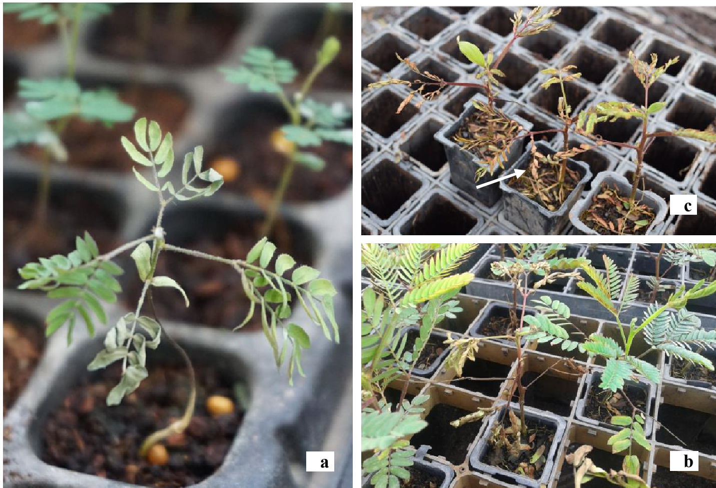


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Fig. 1 Wilt symptoms in the nursery (a) yellowing leaves (b) dry leaves (c).

367 3
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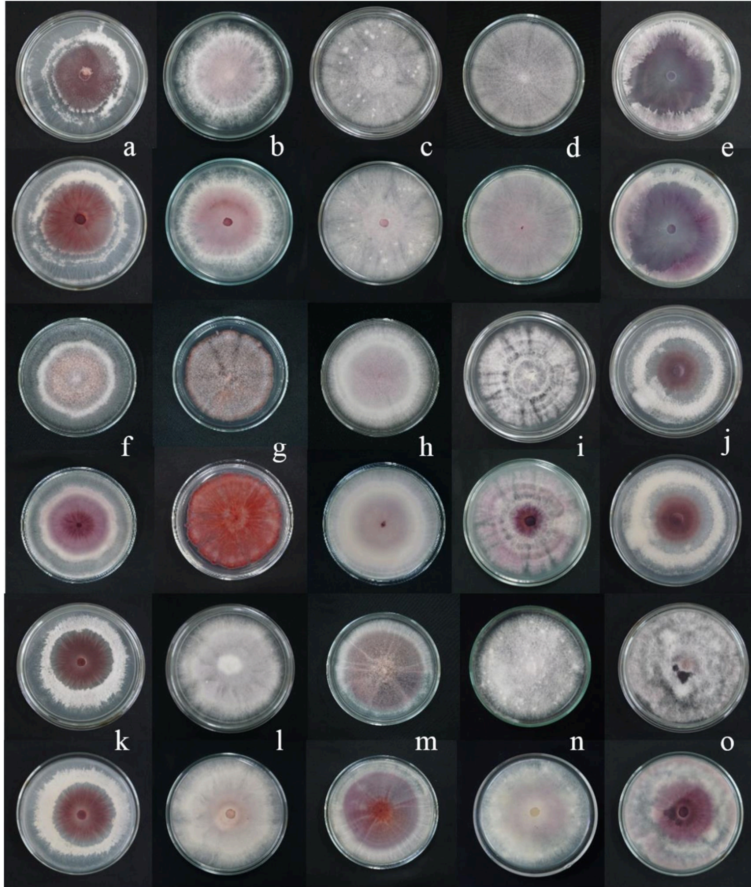
7



368 3
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8 Fig. 2 Colony character on PDA. AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f),

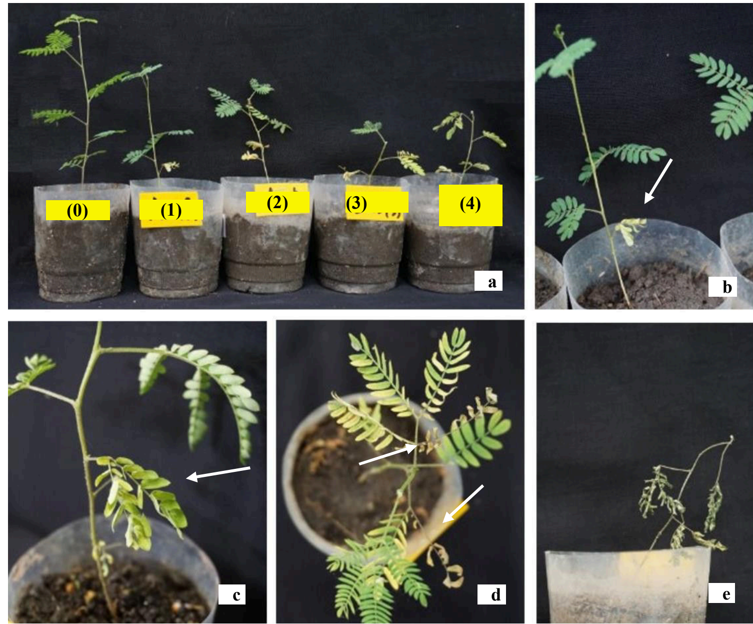
369 3
6
9

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

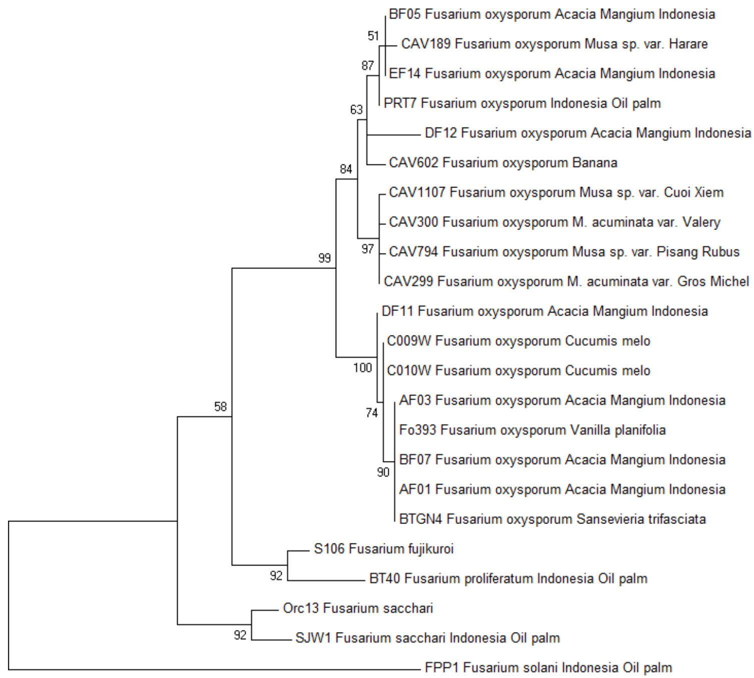
373

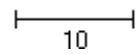


374 Fig. 3 Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial

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

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



377 377 

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.

REVIEWER 3

1 **The Identification and Pathogenicity of *Fusarium oxysporum* causing**

2 **Acacia Seedling Wilt Disease**

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12

13 **Abstract**

14 *Fusarium oxysporum* is an important pathogen in nurseries of commercial acacia in

15 South Sumatra, causing plant mortality of 36.94%. This pathogen induces symptoms of

16 chlorosis in the lower leaves and develops into the shoots; consequently, the plants

17 wither and die. This research aims to identify the pathogenic species causing seedling

18 wilt disease in *Acacia mangium* and its pathogenicity. To achieve this, 15 isolates of *F.*

19 *oxysporum* with varying colony size and color pigment were confirmed by observing

20 the morphological characters and elongation factor 1-α *(tef1-α)* gene sequences. The

21 pathogenicity test showed that all isolates could infect plants with wilt severity reaching

22 80%, and the *Fusarium pathogen* was verified as causing vascular disease.

23 Furthermore, Koch's postulate was confirmed by re-isolating the *F.oxysporum* isolate.

24 The primary source of pathogenic inoculums in commercial nurseries at South Sumatra

25 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.

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

27

28 **INTRODUCTION**

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

41 *F. oxysporum* is a soil-borne pathogen with a very wide host range and is
42 common in various regions (Bayona et al. 2011; Orr and Nelson 2018; Edel-Hermann
43 and Lecomte 2019) including forest and industrial plants (Widyastuti et al. 2013). This
44 pathogen attacks all phases of growth (vegetative and generative) and is able to survive
45 on plant debris for a long period (Postic et al. 2012; Meena and Roy 2020). It causes
46 vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The
47 symptoms involve chlorosis in the leaves, stunted growth, discoloration of the plant's
48 vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al.
49 2019). This species is a dangerous pathogen for plants cultivated both on open land and
50 in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

51 Previous studies have reported seedling wilt disease affecting *Acacia koae* in
52 Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980). Furthermore, *F.*
53 *oxysporum* was reported to have attacked *Acacia nilotica* seedlings in the Green House
54 of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium F.*
55 *oxysporum*, has been found to cause damping-off disease in seedlings six days after
56 germination (Widyastuti et al. 2013). This research aims to identify the pathogenic
57 species causing wilt disease in advanced stage seedlings of *A. mangium* and the
58 disease's pathogenicity.
59

60 **MATERIAL AND METHODS**

61 **Survey and sampling**

62 Soil and diseased plant samples were collected from five commercial company-
63 owned acacia nursery estates with a seedling wilt problem. The diseased plants showed
64 initial symptoms in the form of chlorosis; later, the plants went on to wilt and dry up.
65 The symptoms started from the lower leaves, moving on to the upper leaves and the
66 shoots. Plant samples showing wilting symptoms were collected and stored in a cool
67 box during the process. To determine soil infectivity, acacia seeds were sown using a
68 seedbed, and to accelerate germination, they were soaked in hot water (± 95 ˚C) and left
69 to cool slowly until they reached room temperature (Gardner 1980). Subsequently, the
70 seeds were surface sterilized using 1% sodium hypochlorite for 15 min, and rinsed three
71 times with sterile distilled water. They were then incubated for 2 × 24 h to accelerate
72 the radicula germination. The germinated seeds were planted on infested field nursery
73 medium. The infected seedlings from the field and the infected soil nursery medium
74 were taken and the pathogen was isolated from the plant tissue.
75

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

76 **Fungal isolation**

77 Fungi were isolated from the roots of plants showing the symptoms of seedling
78 wilt, both from the field and the soil infected with pathogens. Then, the root samples
79 were washed under running water, and the surface was sterilized by dipping them in a
80 solution containing 1% sodium hypochlorite for 2 min, rinsing them three times using
81 sterile distilled water, and drying them on filter paper in a laminar airflow (Suwandi et
82 al. 2012). Then, they were planted in a Petri plate containing agar water with 2% (w/v)
83 agar and 0.1% Streptomycin-sulfate and incubated for 2 × 24 h (Gardner 1980; Leslie
84 and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root
85 cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium
86 using the single hyphae method. The isolate results were used for further research.
87

88 **Morphological identification**

89 The initial identification was carried out based on the Leslie and Summerell
90 (2006) method. The observation of cultural characteristics and the morphology of the
91 colony included growth rates and color pigments produced on PDA media. Asexual
92 spores and other structures were observed based on the growth produced on carnation
93 leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX
94 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta,
95 Indonesia). Measurements were carried out on 100 spores of microconidia,
96 macroconidia, and chlamydospores using Image Raster 3.0 software with magnification
97 adjusted to a microscope.
98

99 **Pathogenicity test**

100 A pathogenicity test was carried out on *A. mangium* seedlings 30 days after
101 sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which
102 had previously been sterilized using an autoclave. Fungal isolates were grown in a
103 potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces. The cultures
104 were incubated for three days using a shaker at a speed of 120 rpm to produce large
105 quantities of conidia. Its suspension was inoculated by pouring 1×10^6 cfu g^{-1} soil in a
106 soil medium, while the uninoculated control was watered only with sterile distilled
107 water. Each isolate was inoculated on 10 test plants and the experiment was repeated
108 once. Disease incidence was counted by the number of diseased plants out of the 10
109 tested plants. The severity of the disease was calculated using a score of 0–4, where 0
110 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted,
111 3 = severe wilt, and 4 = dead seedling. The plants were then observed for 1−30 days
112 after inoculation. The difference in disease severity and the area under the disease
113 progress curve (AUDPC) between isolates was calculated through ANOVA and
114 Tukey's HSD test. An analysis was performed using the SAS university edition
115 software package.
116
117 **Molecular identification**
118 Fungal isolates were grown in a liquid medium of PDB on a cultivation bottle
119 containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water). The 3–
120 4-day old isolates on the PDA medium measuring 5 × 5 mm were placed in a PDB
121 liquid medium and incubated for 3–4 days at room temperature. Meanwhile, the fungal
122 mycelium was harvested using vacuum filtration and then it was frozen. The DNA was
123 extracted using the YeaStar Genomic DNA Kit (Zymo Research Corporation,
124 California, USA) following manufacturer's instructions. Its concentration and quality

125 were determined by spectrophotometry using a NanoDrop Spectrophotometry ND-
126 1000 (NanoDrop Technologies, Montchanin, Delaware, U.S.A.) and stored at -20 °C
127 until used. The translation elongation factor 1-α (*tef1*) was amplified using primers EF1
128 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (forward:
129 5′-GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out
130 in 50 µL of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany)
131 (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each
132 primer, and 2 µL of DNA template. The amplification was performed using a PCR
133 Cycler Termal C1000 Touch ™ (Bio- rad, USA). The initial denaturation was
134 performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C, annealing for 40
135 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation step of 5 min at 65
136 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st BASE,
137 Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by
138 comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID
139 databases. To determine the genetic relatedness of *Fusarium oxysporum* from *Acacia*
140 *mangium* with the known *Fusarium* population, the *tef1* sequences were aligned using
141 Clustal-W in MEGA7 and maximum parsimony (MP) analyses were performed. There
142 was a total of 583 positions in the final dataset. All positions containing gaps and
143 missing data were eliminated. The MP tree was obtained using the subtree pruning
144 regrafting algorithm (Nei and Kumar 2000) under MEGA7 (Kumar et al. 2016).
145
146 **RESULTS AND DISCUSSION**
147 **Results**
148 ***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

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

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

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

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

272 through morphological and *tef1* gene sequencing and Koch's postulate. The main
273 source of primary inoculum which causes *Fusarium* wilt disease in South Sumatra
274 comes from a soil seedling medium infested with pathogens.
275
276 **Acknowledgement**

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282 **REFERENCES**

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

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

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

291 Bayona LG, Grajales A, Cardenas ME, Sierra R, Lozano G, Garavito MF, Restrepo S
292 (2011) Isolation and characterization of two strains of *Fusarium oxysporum*
293 causing potato dry rot in Solanum tuberosum in Colombia. Rev Iberoam Micol
294 28(4): 166–172.

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

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

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

303 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–

326 409.

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.

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

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Abstract:	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, the plants wither and die. This research aims to identify the pathogenic species causing seedling wilt disease in A. mangium and to assess its pathogenicity. Total 15 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 were confirmed by re-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 .
Suggested Reviewers:	Achmadi Priyatmojo, Prof. Lecturer, Gadjah Mada University: Universitas Gadjah Mada priyatmojo@ugm.ac.id He had research and Extension Interest in Biology, Ecology and Control of Fungal Soil-borne, Pathogen (Rhizoctonia Species), Plant Growth Promoting Fungi (PGPF), Plant Disease Clinic, Epidemiology of Plant Diseases, Rice Diseases
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MANUSCRIPT REVISED

1 **The Identification and Pathogenicity of *Fusarium oxysporum* causing**

2 **Acacia Seedling Wilt Disease**

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12

13 **Abstract**

14 *Fusarium oxysporum* is an important pathogen in nurseries of commercial acacia in

15 South Sumatra, causing plant mortality of 36.94%. Wilt disease with unknown etiology

16 causes mass mortality in commercial *Acacia mangium* nursery of South Sumatra. This

17 pathogen induces symptoms of chlorosis in the lower leaves and develops into the

18 shoots; subsequentlyconsequently, the plants wither and die. This research aims to

19 identify the pathogenic species causing seedling wilt disease in *A.cacia mangium* and

20 to assess its pathogenicity. TotalTo achieve this, 15 isolates of -*F. oxysporum* with

21 varying colony sizes and color pigments were recovered from symptomatic *A. mangium*

22 seedlings. confirmed by observing the morphological characters and elongation factor

23 1-α (*tef1-α)* gene sequences. The pathogenicity test showed that all isolates could infect

24 plants with wilt severity reaching 80%, and the *Fusarium* pathogen was verified as

25 causing vascular disease. Furthermore, Koch's postulate werewas confirmed by re-

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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 grow~~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 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 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 plantations~~plants~~ (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

51 2020). It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon
52 2017). The symptoms involve chlorosis in the leaves, stunted growth, discoloration of
53 the plant's vascular vessels, and withering, resulting in death (Velarde-Félix et al. 2018;
54 Sun et al. 2019). This species is a dangerous pathogen for plants cultivated both on open
55 land and in greenhouses (Altinok et al. 2018; Velarde-Félix et al. 2018).

56 Previous studies have reported seedling wilt disease affecting *Acacia koa*~~*e*~~ in
57 Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980; Dobbs et al. ~~,~~
58 2020;~~ Gardner, 1980~~)~~(Gardner 1980)~~. Furthermore, *F. oxysporum* was reported to have
59 attacked *Acacia nilotica* seedlings in the Green House of the Forest Research Institute,
60 India (Kapoor et al. 2004). On *A. mangium* in Papua (Indonesia), *F. oxysporum*~~,~~ has
61 been found to cause damping-off disease in seedlings six days after germination
62 (Widyastuti et al. 2013). This research aims to identify the pathogenic species causing
63 wilt disease in advanced stage seedlings of *A. mangium* and the disease's pathogenicity.
64

65 **MATERIAL AND METHODS**

66 **Survey and sampling**

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

Field Code Changed

76 min, and rinsed three times with sterile distilled water. They were then incubated for ~~2~~
77 ~~× 24~~48 h to accelerate the radicul development~~radicula germination~~. The germinated
78 seeds were planted on infested field nursery medium. The infected seedlings from the
79 field and the infested~~infected~~ soil nursery medium were sampled~~taken~~ and the pathogen
80 was also isolated from the plant tissue.
81
82 **Fungal isolation**
83 Fungi were isolated from the roots of plants growing in the field that showed
84 wilt symptoms and also from~~showing the symptoms of seedling wilt, both from the~~
85 ~~field and the~~ soil infested~~infected~~ with pathogens. Then, the root samples were washed
86 under running water, and the surface was sterilized by dipping them in a solution
87 containing 1% sodium hypochlorite for 2 min, rinsing them three times using sterile
88 distilled water, and drying them on filter paper in a laminar airflow (Suwandi et al.
89 2012). Then, they were planted in a Petri plate containing agar water with 2% (w/v)
90 agar and 0.1% Streptomycin-sulfate and incubated for ~~2 × 24~~48 h (Gardner 1980; Leslie
91 and Summerell 2006; Suwandi et al. 2012). The mycelium that grew from the root
92 cuttings was transferred to potato dextrose agar (PDA) (Merck, Germany) medium
93 using the single hyphae method. The isolate results were used for further research.
94
95 **Morphological identification**
96 The initial identification was carried out based on the Leslie and Summerell
97 (2006) method. The observation of cultural characteristics and the morphology of the
98 colony included growth rates and color pigments produced on PDA media. Asexual
99 spores and other structures were observed based on the growth produced on carnation
100 leaf agar (CLA) medium. These were made under a light microscope (OLYMPUS CX

101 23) at 1000 × magnification with a camera (Optilab Advance Plus, Yogyakarta,
102 Indonesia). Measurements were carried out on 100 spores of microconidia,
103 macroconidia~~,~~ and chlamydospores using Image Raster 3.0 software with magnification
104 adjusted to a microscope.
105
106 **Pathogenicity test**
107 A pathogenicity test was carried out on *A. mangium* seedlings 30 days after
108 sowing. This plant was grown in plastic pots containing peat soil medium (200 g) which
109 had previously been sterilized using an autoclave. Fungal isolates were grown in a
110 potato dextrose broth (PDB) medium by placing 5 × 5 mm agar pieces of the fungal
111 colony in the broth. The cultures were incubated for three days using a shaker at a speed
112 of 120 rpm to produce large quantities of conidia. This~~Its~~ suspension was used as
113 inoculum~~inoculated~~ by pouring 1 × 10^6 cfu g^{-1} soil in a soil medium, while the
114 uninoculated control was watered only with sterile distilled water. Each isolate was
115 inoculated into the soil of~~on~~ 10 test plants and the experiment was repeated once.
116 Disease incidence was counted by the number of diseased plants out of the 10 tested
117 plants. The severity of the disease was calculated for each seedling using a score of 0–
118 4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and
119 slightly wilted, 3 = severe wilt, and 4 = dead seedling. The plants were then observed
120 for 1−30 days after inoculation. The difference in disease severity and the area under
121 the disease progress curve (AUDPC) between isolates was calculated through ANOVA
122 and Tukey's HSD test. An analysis was performed using the SAS university edition
123 software package.
124
125 **Molecular identification**

126 Seven representatives Ffungal isolates with distinct morphological

127 characteristics were selected and grown in a liquid medium of PDB inon a cultivation

128 bottle containing 50 mL sterile PDB (200 g potato; 20 g glucose; 1 L distilled water)

129 section of. The 3–4-day old culturesisolates on the PDA medium measuring 5 × 5 mm

130 were placed in a PDB liquid medium and incubated for 3 – 4 more days at room

131 temperature. Meanwhile, the fungal mycelium was harvested using vacuum filtration

132 and then it was frozen. The DNA was extracted using the YeaStar Genomic DNA Kit

133 (Zymo Research Corporation, California, USA) following manufacturer's instructions.

134 Its concentration and quality were determined by spectrophotometry using a NanoDrop

135 Spectrophotometry ND - 1000 (NanoDrop Technologies, Montchanin, Delaware,

136 U.S.A.) and stored at -20 °C until used. The translation elongation factor 1-α (*tef1*) was

137 amplified using primers EF1 (forward: 5′-ATGGGTAAGGAAGACAAGAC-3′) and

138 EF2 (reverseforward: 5′ -GGAAGTACCAGTGATCATGTT -3′) (O'Donnell et al.

139 1998). PCR was carried out in 50 µL of the reaction mixture containing 20 µL Master

140 Mix (Eppendorf, Germany) (1.25 GoTaq DNA polymerase, 0.2 µM of each dNTP, 2 ×

141 PCR buffer), 1 µL of each primer, and 2 µL of DNA template. The amplification was

142 performed using a PCR Cycler Termal C1000 Touch ™ (Bio-rad, USA). The initial

143 denaturation was performed for 2 min at 95 °C followed by 30 cycles for 20 s at 95 °C,

144 annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation

145 step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were

146 analyzed at 1st BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained

147 was determined by comparing the GenBank (http://www.ncbi.nlm.nih.gov) and

148 FUSARIUM-ID databases. To determine the genetic relatedness of *Fusarium*

149 *oxysporum* from *A.cacia mangium* with the known *Fusarium* population, the *tef1*

150 sequences were aligned using Clustal-W in MEGA7 and maximum parsimony (MP)

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151 analyses were performed. There was a total of 577~~83~~ positions in the final dataset. All
152 positions containing gaps and missing data were eliminated. The MP tree was obtained
153 using the subtree pruning regrafting algorithm (Nei and Kumar 2000) under MEGA7
154 (Kumar et al. 2016).
155

156 **RESULTS AND DISCUSSION**

157 **Results**

158 ***Disease symptoms and wilt incidences***

159 Surveys on seedling wilt were carried out in six acacia nursery locations in
160 commercial companies, with five sites in the Air Sugihan area and one site in Lebong
161 Hitam (Table 1). The incidence of disease varied between locations from as low as
162 6.0~~5.6~~% up to 36.9%. The early symptoms of seedling wilt started from the lower leaves
163 as they turned yellow, and then black, dried out, fell and the plant died. Some symptoms
164 began with yellowish discoloration of the leaves, which then wilted, and some had no
165 discoloration but immediately began with wilting (Fig. 1). Observations were made by
166 planting acacia on used soil medium from the commercial nursery, and the results
167 showed that 56.25% of the seedlings were attacked. In the commercial nursery, this
168 disease was found to attack acacia seedlings at an average age of more than 1 month
169 after germination and before the formation of phyllodes or false leaves.
170

171 ***Morphological characteristic***

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

175 produced several color pigments, such as purple, pale purple and reddish-pink, with air
176 hyphae (Fig. 2). These isolates showed varied growth rates of the colony, with the
177 fastest on BF05 and FF15 isolates (11.38 mm/day). BF06 showed the slowest colony
178 growth rate (7.46 mm/day) compared to the others (Fig. 3Table 3). They all produce
179 many microconidia on the false heads of monophialides. Hyaline microconidia are oval,
180 elliptical, reniform to allantoid with an average size of 5.5 ± 1.0 µm × 2.8 ± 0.5 µm to
181 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have 0 to 1 septum, but generally 0. The hyaline
182 macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ± 0.5 µm
183 to39.7 ± 5.9 × 3.8 ± 0.4 µm, and have 3 to 7 septa, but generally 3. The chlamydospores
184 produced singly/in pairs at the terminal/intercalary have an average diameter of 6.4 ±
185 0.6 µm to 10.0 ± 2.5 µm (Table 34). Based on the morphological characteristics of the
186 PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as
187 described by Leslie and Summerell (2006).
188
189 ***Molecular characteristics***
190 The molecular identification achieved by sequencing the *tef1* gene was
191 amplified using primers EF1 and EF2 against seven selected isolates. The sequence
192 data was determined by making a comparison with those existing in Genbank through
193 the BLAST and FUSARIUM-ID programs. Based on this, the sequences were
194 confirmed as *F. oxysporum* with 84.9−100% similarity to Genbank and 97.3−99.7%
195 with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8, retention index
196 0.9 and the composite index 0.87) grouped all *Fusarium* isolates within the *F.*
197 *oxysporum* species complex. Isolates were clustered within four clades (Fig. 54). The
198 first clade consisted of BF05 and EF14, and *F. elaeidis* (MH484961.1) from *Elaeis* sp.
199 The first clade consisted of AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum*

200 ~~from *Sanseviera trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*).~~ The
201 second clade consisted of single isolates from *A. mangium* (DF11), ~~and~~ *F. oxysporum*
202 (C009W and C010W) from *Cucumis melo*, and *F. triseptatum* (MH484964.1) from
203 *Ipomoea batatas*. ~~The third consisted of DF12 and an isolate of *F. oxysporum* from~~
204 ~~*Musa* sp. The fourth clade consisted of BF05 and EF14, and *F. oxysporum* (CAV189)~~
205 ~~from *Musa* sp. var. Harare.~~ The third ~~first~~ clade consisted of AF01, AF03 and BF07
206 along with BTGN4 (*F. oxysporum* from *Sanseviera trifasciata*) and FO393 (*F.*
207 *oxysporum* from *Vanilla planifolia*). The fourth consisted of DF12 and an isolate of *F.*
208 *oxysporum* from *Musa* sp. *Tef1* sequences of isolates within third and fourth clade were
209 separated from all 15 cryptic taxa of *F. oxysporum* species complex as described by
210 ~~(~~Lombard et al.~~, Lamprecht, & Crous,~~ (2019).
211

212 ***Pathogenicity tests***

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

225 higher compared to the control. The AF02, BF06, CF10, DF11, DF12 and DF13 isolates
226 caused disease severity and disease progress and their AUDPC values were
227 significantly higher than other isolates and the control (Table 4). Concerning disease
228 incidence, the *Fusarium* isolates presented a high variation in percentage of disease
229 incidence, ranging from 50 − 100%. These~~This~~ isolates also induced wilting
230 severities~~severity~~ ranging from 1.2−3.2. The highest disease incidence was caused by
231 isolate BF06 (100%), while the highest disease severity was caused by isolate DF11
232 (3.2). Based on disease severity, the isolates were grouped into three categories of
233 virulence: high (score 2−4), moderate (score 1.2−2), and low score (0−1.2) virulence
234 (Dubey et al. 2010). BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11
235 were included in the high virulence group (2.1−3.2), while FF15, BF09, BF07, AF03,
236 and BF05 were in the moderate (1.6–2.0), and only EF14 was in the low. We observed
237 that this pathogen causes vascular disease, where the pathogen could be isolated from
238 the basal stem up to the shoots. To confirm the Koch's Postulates test, the pathogens
239 were all re-isolated (100%) from infected plants and it was confirmed that the
240 morphological characters of the pathogen were the same as the inoculated *Fusarium*
241 isolates. The pathogen was not isolated from the uninoculated control plants.
242

243 **Discussion**

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

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

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

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

266 *F. oxysporum* was determined based on morphological identification, both
267 visually and microscopically. Visually, the fungus produces pale purple to pale pink
268 pigments on a PDA medium. Microscopically, the shape of its reproductive structure is
269 the same as *F. oxysporum,* generally exhibiting a short monophialide (Leslie and
270 Summerell 2006). The analysis of the *tef1* gene sequences confirmed that the disease-
271 causing pathogenic species was *F. oxysporum*. These showed that *F. oxysporum*, which
272 causes acacia seedlings to wilt, is the that most similar to the *F. oxysporum* species
273 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.

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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. J~~ournal of~~ Phytopathol~~ogy,~~ 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. Eur~~opean~~ J~~ournal of~~ Plant Pathol~~ogy,~~ 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. Rev~~ista~~ Iberoam~~ericana de~~ Micol~~ogía,~~ 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)~~.~~ C~~c~~urrent status of *Fusarium oxysporum* f~~F~~ormae s~~S~~peciales and r~~R~~aces. J Phytopathol~~ogy,~~ 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.

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

329 Gordon~~,~~ T.~~ ~~R~~.~~ (2017)~~.~~ *Fusarium oxysporum* and the Fusarium Wilt Syndrome.~~ The~~

330 Annu~~al~~ Rev~~iew of~~ Phytopathol~~ogy,~~ 55:~~,~~ 23–39.

331 Hedge~~,~~ M~~.~~, Palanisamy~~,~~ K~~.~~,~~ &~~ Yi~~,~~ J.~~ ~~S~~.~~ (2013)~~.~~ *Acacia mangium* Willd .~~—~~ A Fast

332 Growing Tree for Tropical Plantations. J~~ournal of~~ For~~est~~ Sci~~ence,~~ 29(1):~~,~~ 1–14.

333 Jiménez-Díaz~~,~~ R.~~ ~~M~~.~~, Castillo~~,~~ P~~.~~, Jiménez-Gasco~~,~~ M.~~ ~~del-M~~.~~, Landa~~,~~ B.~~ ~~B~~.~~,~~ &~~ Navas-

334 Cortés~~,~~ J.~~—~~A~~.~~ (2015)~~.~~ Fusarium wilt of chickpeas: Biology, ecology and

335 management. Crop Protection~~,~~ 73:~~,~~ 16–27.

336 ~~https://doi.org/10.1016/j.cropro.2015.02.023~~

337 Kapoor~~,~~ S~~.~~, Harsh~~,~~ N.~~ ~~S.~~ ~~K~~.~~,~~ &~~ Sharma~~,~~ S.~~ ~~K~~.~~ (2004)~~.~~ A new wilt disease of *A~~a~~cacia*

338 *nilotica* caused by *F~~f~~usarium oxysporum*~~.pdf~~. Journal of Tropical Forest Science~~,~~

339 16(4):~~,~~ 453–462.

340 Kee~~,~~ Y.~~—~~J~~.~~, Zakaria~~,~~ L~~.~~,~~ &~~ Mohd~~,~~ M.~~—~~H~~.~~ (2020)~~.~~ Morphology, phylogeny and

341 pathogenicity of Fusarium species from *Sansevieria trifasciata* in Malaysia. Plant

342 Pathol~~ogy,~~ 69(3):~~,~~ 442–454. ~~https://doi.org/10.1111/ppa.13138~~

343 Koyyappurath~~,~~ S~~.~~, Atuahiva~~,~~ T~~.~~, Le Guen~~,~~ R~~.~~, Batina~~,~~ H~~.~~, Le Squin~~,~~ S~~.~~, Gautheron~~,~~ N~~.~~,

344 ~~…~~Grisoni~~,~~ M~~.~~ (2016)~~.~~ *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal

345 agent of root and stem rot of vanilla. Plant Pathol~~ogy,~~ 65(4):~~,~~ 612–625.

346 ~~https://doi.org/10.1111/ppa.12445~~

347 Kumar~~,~~ S~~.~~, Stecher~~,~~ G~~.~~,~~ &~~ Tamura~~,~~ K~~.~~ (2016)~~.~~ MEGA7: Molecular Evolutionary

348 Genetics Analysis version 7.0 for bigger datasets. Mol~~ecular~~ Biol~~ogy and~~

349 Evol~~ution,~~ 33(7):~~,~~ 1870–1874. ~~https://doi.org/10.2166/nh.2003.0008~~

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

351 Blackwell Publishing, Oxford.~~.~~

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

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

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

359 Meena~~,~~ R.~~ ~~P~~.~~, ~~&~~ Roy~~,~~ S~~.~~ (2020)~~.~~ Morphological and molecular characterization of
360 *Fusarium* sp. causing wilt disease of isabgol (*Plantago ovata* Forsk.) and its
361 management strategies. Journal of Applied Research on Medicinal and Aromatic
362 Plants~~, (July 2019), 100244. https://doi.org/10.1016/j.jarmap.2020.100244~~
363 100244.

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

366 O’Donnell~~,~~ K~~.~~, Kistler~~,~~ H.~~ ~~C~~.~~, Cigernik~~,~~ E~~.~~, ~~&~~ Ploetz~~,~~ R.~~ ~~C~~.~~ (1998)~~.~~ Multiple
367 evolutionary origins of the fungus causing Panama disease of banana : Concordant
368 evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Sci. USA~~,~~
369 95:~~,~~ 2044–2049.

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

373 Postic~~,~~ J~~.~~, Cosic~~,~~ J~~.~~, Vrandecic~~,~~ K~~.~~, Jurkovic~~,~~ D~~.~~, Saleh~~,~~ A.~~ ~~A~~.~~, ~~&~~ Leslie~~,~~ J.~~ ~~F~~.~~ (2012)~~.~~
374 D~~d~~iversity of F~~f~~usarium species isolated from weeds and plant debris in Croatia.
375 J~~ournal of~~ Phytopathol~~ogy,~~ 160:~~,~~ 76–81. ~~https://doi.org/10.1111/j.1439-~~
376 ~~0434.2011.01863.x~~

377 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)~~.~~ C~~C~~ommon spear rot of oil palm in Indonesia ~~Common Spear Rot of Oil Palm in Indonesia~~. Plant Dis~~ease,~~ 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. Can~~adian~~ J~~ournal of~~ Plant Pathol~~ogy,~~ 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.

Table Revised Click here to access/download;Table;Table Rev1.docx

Table 1 Disease incidence in the commercial nursery fields of *Acacia 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	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

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

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

399 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

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

401 test at 5%.

Table 34 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

404 Table ~~4~~5 Incidence, severity and progression of wilt disease in one-month-old acacia seedlings

405 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

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

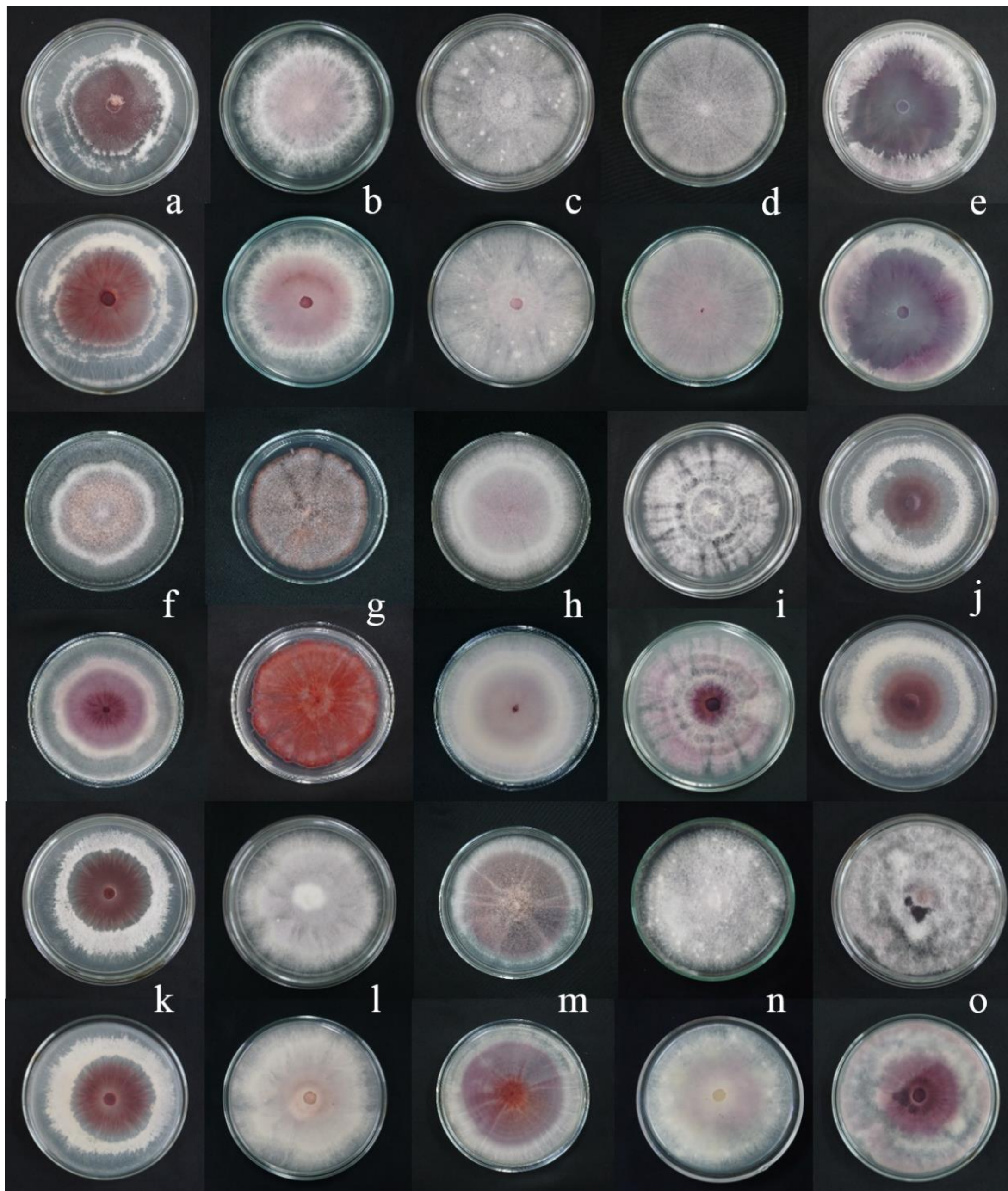
407 test at 5%.

Figure Revised Click here to access/download;Figure;Figure Rev1.docx

408

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

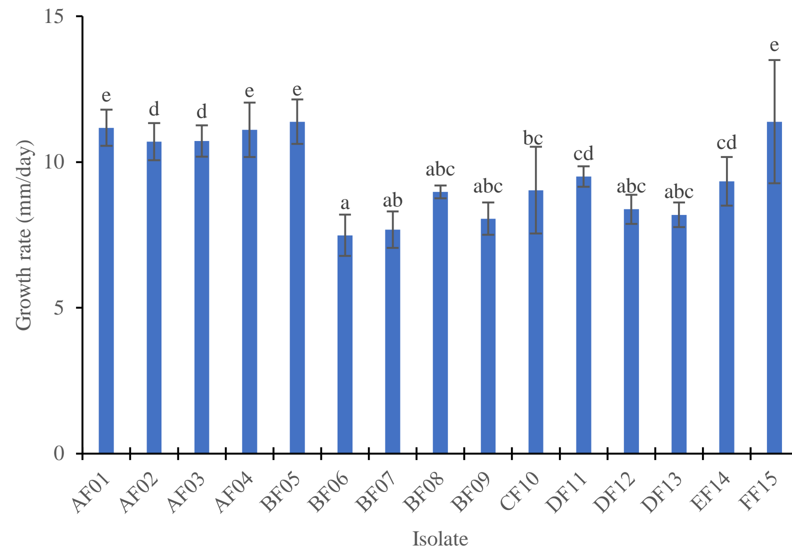
410



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

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

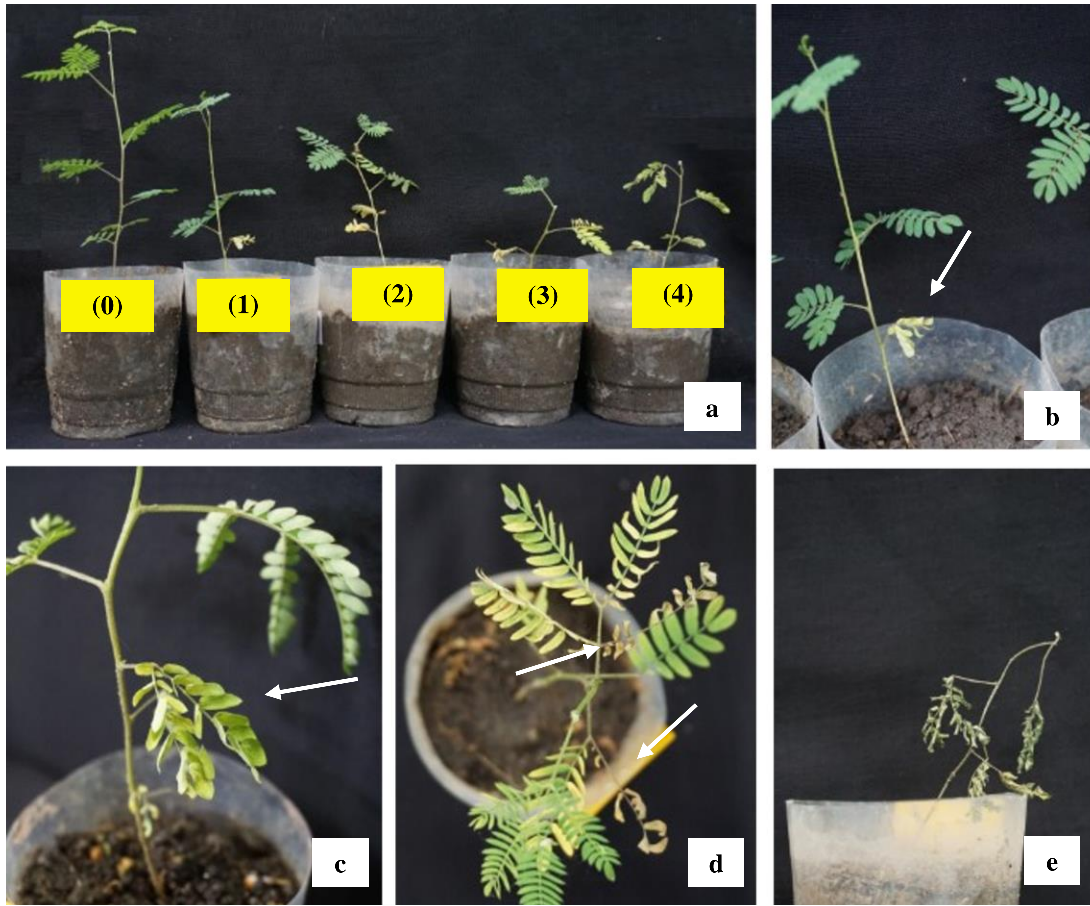
413 Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).



414

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

416

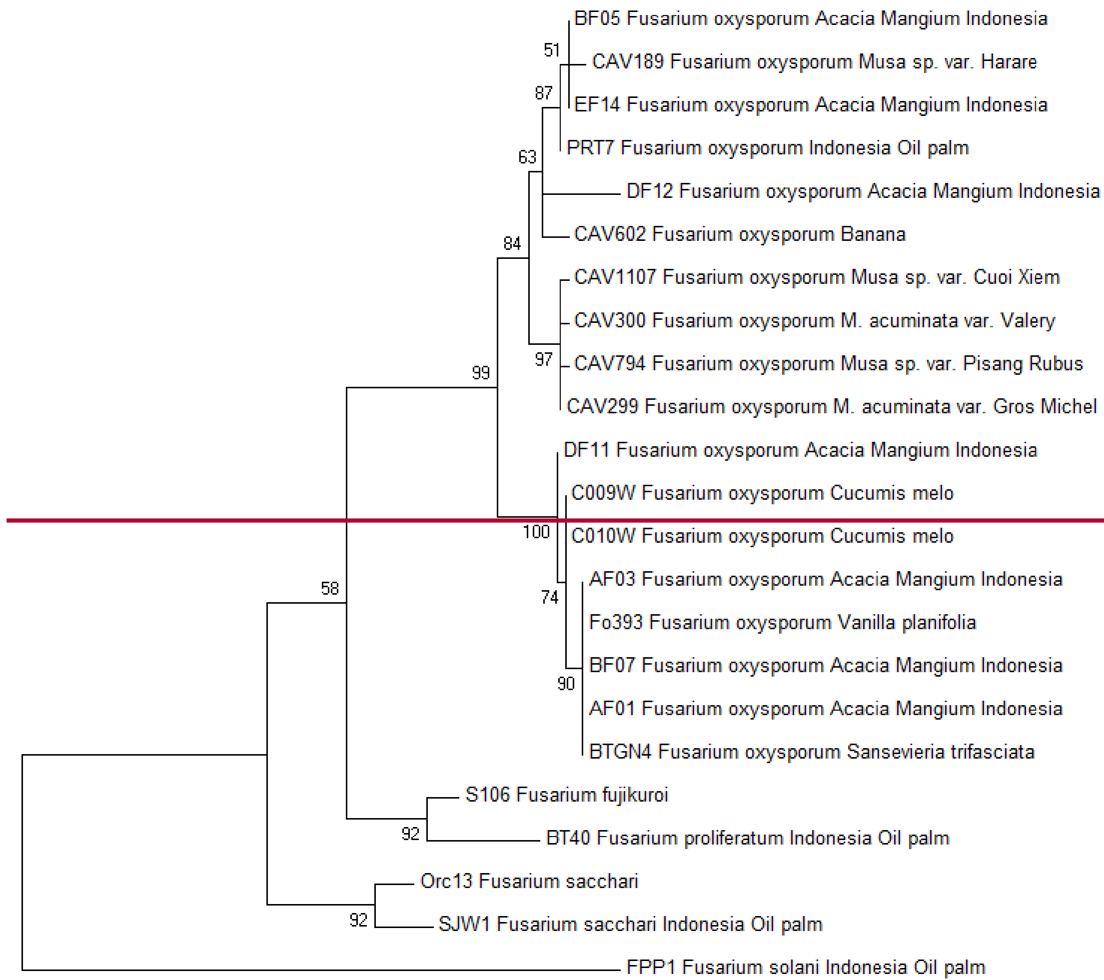


417

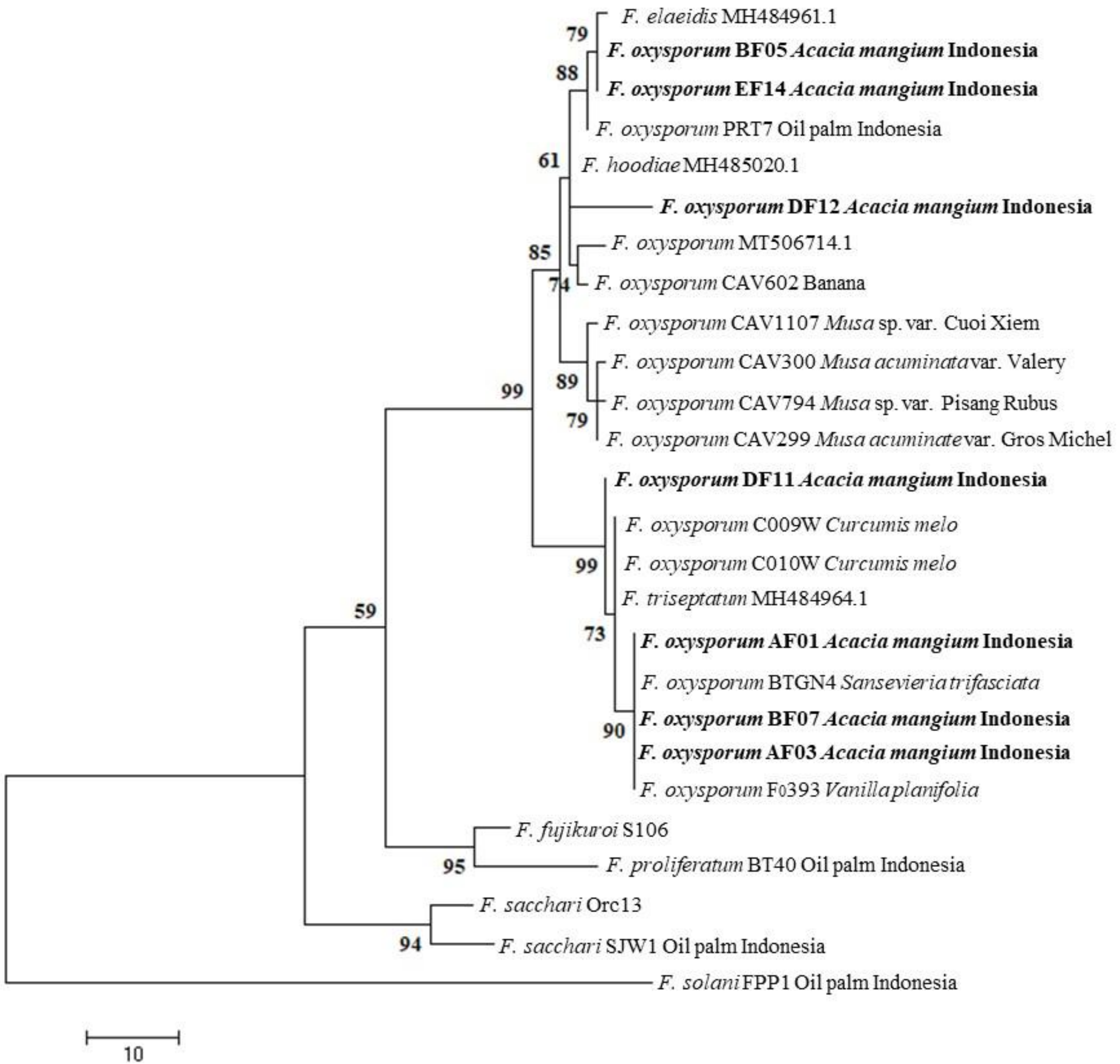
418 Fig. 43 Disease severity rate. From left: healthy plant to 100% wilted leaves (scale 0-4) (a). Initial

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

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



421



422

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

PROOFREAD AND PUBLISHING PROCESS

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

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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

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

https://drive.google.com/file/d/1VdfszkSNj8YZP_8ufFAo1pa43MFGFfGo/view?usp=sharing

On Sun, Apr 25, 2021 at 9:51 AM 徐涛 <tao.xv@nefu.edu.cn> wrote:
[Quoted text hidden]

徐涛 <tao.xv@nefu.edu.cn> Fri, May 7, 2021 at 9:22 AM
To: "a. muslim unsri" <a_muslim@unsri.ac.id>

Dr. Ahmad Muslim,

I'm glad to hear from you. But I can't open the link website and .bin file you provided. Please send the file--JFR-D-21-00042TaoRevised.docx as attachment to me again. Thanks.

Kind regards,

Tao Xu

> -----原始邮件-----
> **发件人:**"a. muslim unsri" <a_muslim@unsri.ac.id>
> **发送时间:**2021-05-07 08:46:02 (星期五)
> **收件人:** "徐涛" <tao.xv@nefu.edu.cn>
> **抄送:**
> **主题:** Re: The corrected version of manuscript JFR-D-21-00042, Journal of Forestry Research
> [Quoted text hidden]

[Quoted text hidden]

PROOFREAD

393 **The Iidentification and Ppathogenicity of *Fusarium oxysporum* causing**

394 **Aacacia Ssseedling Wwilt Ddisease**

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

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403

404 **Abstract**

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

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

417

418 **IntroductionNTRODUCTION**

419 Black wattle (*Acacia mangium* Willd.) is a floweringgreen leafy plantTree species native to Papua,

420 West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in

421 Australia. Under favorable environmental conditions, this species can grow, up to 30 m with a

422 diameter of 50 cm. It is cultivated on industrial forest plantations by large companies for itsbecause

423 it gives high levels of good quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the

424 total land area controlled by industrial plantation forest companies in Indonesia wasere 8.67

425 million hectares, with 81.30% being used for plant cultivation, including *A. mangium*.

426 Furthermore, In addition, in 2017, this plantspecies produced the most logs of any other species,

427 reaching 77.55% from a log total of 40,628.878 m³ (Statistics Indonesia 2018).

428 TheA majorin problem faced in its cultivation is an unknown cause of seedling wilt disease.

429 It occurs during the initial stages of plant growth and, if ignored, it can spread widely in a

430 plantation.

431 *Fusarium oxysporum F. oxysporum* Snyder & Hansen is a soil-borne pathogen with a very

432 wide host range of hosts and is common in various regions of the world (Bayona et al. 2011; Orr

433 and Nelson 2018; Edel-Hermann and Lecomte 2019), including forests and industrial plantations

434 (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and regenerative)

435 and is able to survives on plant debris for a long periods (Postic et al. 2012; Meena and Roy 2020).

436 It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The

437 symptoms involve chlorosis inof the leaves, stunted growth, discoloration of the plant's vascular

438 vessels, and withering, resulting in death (Velarde-Félix et al. 2018; Sun et al. 2019). This species

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439 is a dangerous pathogen for plants cultivated both on open land and in greenhouses (Altinok et al.
440 2018; Velarde-Félix et al. 2018).

441 Previous studies have reported seedling wilt disease affecting *A.cacia koa* A. Gray in
442 Hawaii caused by *F. oxysporum* f. sp. *koae,* f. sp. nov. (Gardner 1980; Dobbs et al. 2020;).
443 Furthermore, *F. oxysporum* was reported to have attacked *A.cacia nilotica* (L.) P. J. H. Hurter &
444 McNabb seedlings in the GgreenhouseHouse of the Forest Research Institute, India (Kapoor et al.
445 2004). On *A. mangium* iIn Papua (Indonesia), *F. oxysporum* has been found to cause damping-off
446 disease inon *A. mangium* seedlings six days after germination (Widyastuti et al. 2013). This
447 research aims to identify the pathogenic species causing wilt disease in advanced stage seedlings
448 of *A. mangium* and the disease's pathogenicity.

449

450 **Methods and materials ATERIAL AND METHODS**

451 **Survey and sampling**

452 Soil and diseased plant samples were collected from six commercial, company-owned acacia
453 nurseriesy estates with a seedling wilt problem. The diseased plants showed initial symptoms in
454 the form of chlorosis; later, the plants went on to wilted and driedy up. The symptoms started from
455 the lower leaves, moving on to the upper leaves and the shoots. Plant sSamples showing wilting
456 symptoms were collected and stored in a cool box. during the process. To determine soil
457 infectivity, acacia seeds were sown usingon a seedbed, and to accelerate germination, they were
458 first soaked in hot water (± 95 ˚C) and left to cool slowly until they reached room temperature
459 (Gardner 1980). Subsequently, the seeds were surface sterilized using 1% sodium hypochlorite for
460 15 min, and rinsed three times with sterile distilled water. They were then incubated for 48 h to
461 accelerate the radicule development. The germinated seeds were planted on infested field nursery

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462 soil.medium. The infected seedlings from the field and the infectedsted soil nursery soil medium

463 were sampled and the pathogen was also isolated from the plant tissue.

464

465 **Fungal isolation**

466 Fungi were isolated from the roots of plants growing in the field that showed wilt symptoms of

467 wilt and also from soil infectedsted with pathogens. Then, tThe root samples were then washed

468 under running water, and the surface was sterilized by dipping them in a solution containing 1%

469 sodium hypochlorite for 2 min, rinsing them three times inusing sterile distilled water, and drying

470 them on filter paper in a laminar airflow (Suwandi et al. 2012). Then, tThey were then laid out on

471 planted in a Petri plate ofcontaining agar water with 2% (w/v) agar and 0.1% Sstreptomycin-

472 sulfate, and incubated for 48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012).

473 The mycelium that grew from the root cuttings was transferred to potato dextrose agar (PDA)

474 (Merck, Germany) medium using the single hyphae method. The isolate results were used for

475 further research.

476

477 **Morphological identification**

478 The initial identification was carried out based on the Leslie and Summerell (2006). method. The

479 observation of cultural characteristics and the morphology of the colony included growth rates and

480 color pigments produced on PDA media. Asexual spores and other structures were observed based

481 on the growth produced on carnation leaf agar (CLA) medium. These were made under a light

482 microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus,

483 Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia, and

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

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484 macroconidia~~,~~ and chlamydospores using Image Raster 3.0 software with magnification adjusted

485 to a microscope.

486

487 **Pathogenicity test**

488 ~~A pathogenicity~~This test was carried out on *A. mangium* seedlings 30 days after sowing~~. This plant~~

489 ~~was grown~~ in plastic pots containing 200 g peat soil ~~medium (200 g)~~ which had previously been

490 sterilized. ~~using an autoclave.~~ Fungal isolates were grown ~~i~~on a potato dextrose broth (PDB)

491 medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were

492 incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia.

493 This suspension was used as inoculum by pouring 1 × 10^6 cfu g^{-1} (colony forming unit/g) ~~soil~~ ~~i~~on

494 a soil medium~~,~~ while the uninoculated control was ~~watered only with~~ sterile distilled water. Each

495 isolate was inoculated into the soil of 10 test plants and the experiment ~~was~~ repeated once. Disease

496 incidence was counted ~~by~~as the number of diseased plants out of ~~the~~ 10. ~~tested plants.~~ The severity

497 of the disease was calculated for each seedling using a score of 0–4, where 0 = no disease/healthy

498 seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead

499 seedling. The plants were ~~then~~ observed ~~for 1–~~over 30 days after inoculation. The difference in

500 disease severity and the area under the disease progress curve (AUDPC) between isolates was

501 calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS

502 university edition software package.

503

504 **Molecular identification**

505 Seven representative~~s~~ fungal isolates with distinct morphological characteristics were selected and

506 grown ~~in a liquid medium of PDB~~ in a cultivation bottle containing 50 mL sterile PDB (200 g

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507 potato; 20 g glucose; 1 L distilled water). ~~s~~Sections of 3–4- day--old cultures on the PDA medium,

508 measuring 5 × 5 mm, were placed ~~i~~on a PDB liquid medium and incubated for 3–4 additional~~more~~

509 days at room temperature. ~~Meanwhile, t~~The fungal mycelium was harvested using vacuum

510 filtration and ~~then it was~~ frozen. ~~The~~ DNA was extracted using the YeaStar Genomic DNA Kit

511 (Zymo Research Corporation, Irvine, CA,~~California,~~ USA) following manufacturer's instructions.

512 Its concentration and quality were determined by spectrophotometry using a NanoDrop

513 Spectrophotometry ND‑1000 (NanoDrop Technologies, Montchanin, DE,~~elaware,~~ U.S.A.) and

514 stored at-20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers

515 EF1 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (reverse:

516 5′-GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL

517 of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA

518 polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA

519 template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio‑

520 rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for

521 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation

522 step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st

523 BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by

524 comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To

525 determine the genetic relatedness of *Fusarium oxysporum* from *A. mangium* with the known

526 *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum

527 parsimony (MP) analyses were performed. There was a total of 577 positions in the final dataset.

528 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 nurseriesy locations inof 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 fromas 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 56.255% 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.

Morphological characteristics

Fifteen isolates similar to *F. oxysporum* were takenisolated 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

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552 showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.4~~38~~ mm/day). BF06 had~~showed~~ the slowest ~~colony~~ growth rate (7.5~~46~~ mm/day) ~~compared to the others~~ (Fig. 3). They all produced ~~many~~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 ± 4.1µm × 3.0 ± 0.3 µm, and have zero~~0~~ to ~~1~~one 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 three~~3~~ to seven~~7~~ septa~~,~~ but usually~~generally~~ ~~3~~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).

563

564 ***Molecular characteristics***

565 ~~The m~~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) grouped all *Fusarium* isolates within the *F. oxysporum* species complex. Isolates were clustered within four clades (Fig. 5). The first clade comprised~~nsisted 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

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575 contained~~consisted of~~ AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera*
576 *trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The fourth included~~consisted 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,~~ curving~~ed~~
585 upwards, turning~~ed~~ brown to black, drying and ~~, became dry, and~~ ~~fell~~falling from the plant. This
586 ~~symptom~~ progresses to the top of the plant, causing it to wither and die. ~~Furthermore~~In 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 showed~~experienced~~ 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 to~~Concerning~~ disease incidence, the *Fusarium* isolates
595 had~~presented~~ a high variation in percentage of disease incidence, ranging from 50−100%. These
596 isolates also induced wilting severities of~~ranging 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

598 (3.2). Based on disease severity, the isolates were grouped into three categories of virulence: high
599 (score 2.0−4.0), moderate (score 1.2−2.0), and low score (0−1.2) virulence (Dubey et al. 2010).
600 BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11 were included in the high
601 virulence group (2.1−3.2), while FF15, BF09, BF07, AF03, and BF05 were in the moderate (1.6−
602 2.0), and only EF14 had awas in the low score. We observed that this pathogen causes vascular
603 disease, where the pathogen could be isolated from the basal stem up to the shoots. To confirm the
604 Koch's Postulates test, the pathogens were all re-isolated (100%) from infected plants and it was
605 confirmed that the morphological characters of the pathogen were the same as the inoculated
606 *Fusarium* isolates. –The pathogen was not isolated from the uninoculated control plants.
607
608 **Discussion**
609 ———This research reports that *F Fusarium. oxysporum* washas been identified for the first time
610 as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to
611 previous studies, *F. oxysporum* has beenwas reported to beas the cause of seedling wilt in *A. koa*
612 in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen
613 was reported to attack acacia germinated acacia seeds, resulting in damping-off, and the
614 identification of this was made solely based on morphological characters (Widyastuti et al. 2013).
615 OurThis research confirms that *F. oxysporum*, identified by morphological and molecular
616 methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu
617 (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana* Lamb.
618 The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms
619 with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2,
620 respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in

621 India with diseases severity of 16.9~~86~~% (Kapoor et al. 2004), on *A. koa* in Hawaii with 85.0%
622 severity (Gardner 1980), and damping-off on *A. mangium* in Papua, Indonesia with unknown
623 disease severity (Widyastuti et al. 2013).

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

629 *F. oxysporum* was ~~determined~~ determined based on morphological identification, both
630 visually and microscopically. Visually, the fungus produces pale purple to pale pink pigments on
631 a PDA medium. Microscopically, the shape of its reproductive structure is the same as *F.*
632 *oxysporum,* generally exhibiting a short monophialide (Leslie and Summerell 2006). The analysis
633 of the *tef1* gene sequences confirmed that the disease-causing pathogenic species was *F.*
634 *oxysporum*. These showed that *F. oxysporum*, which causes acacia seedlings to wilt, is the ~~that~~
635 most similar to the *F. oxysporum* species complex from *Elaeis* sp. (*F. elaeidis*) (Lombard et al.
636 2019), *S. trifasciata* (Kee et al. 2020), *V. planifolia* (Koyyappurath et al. 2016), *C. melo* (Bakar
637 and Mohd 2019).

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

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

644 that clonal dispersion occurred via the nursery medium to the commercial field nursery as shown
645 by isolates within the first clade of the *tef1* sequences originating from separate locations. Jiménez-
646 Díaz et al. (2015) reported that the main source of primary inoculum that causes *Fusarium* wilt
647 comes from infe~~c~~sted soil and that pathogens can survive a long ~~time~~periods in the soil, and~~. The~~
648 ~~pathogens can also survive~~ in other plant debris (Postic et al. 2012; Altinok 2013)
649

650 **Conclusion**

651 This study ~~present~~is the first report of *F. oxysporum* as a causal agent of *A. mangium* seedling wilt
652 in South Sumatra, Indonesia. The pathogen was confirmed through morphological and *tef1* gene
653 sequencing and Koch's postulate. The main source of a primary inoculum which causes *Fusarium*
654 wilt disease in South Sumatra comes from ~~a~~seedling soils ~~seedling medium~~ infe~~c~~sted with
655 pathogens.
656

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

657 **Acknowledgement**

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

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662 ~~REFERENCES~~

663 References

664 Altinok HH (2013) Fusarium species isolated from common weeds in eggplant fields and
665 symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161:
666 335–340.

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

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

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

675 Dobbs JT, Kim MS, Dudley NS, Klopfenstein NB, Yeh A, Hauff RD, Stewart JE (2020) Whole
676 genome analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) and the
677 development of molecular tools for early detection and monitoring. BMC Genomics 21: 1–
678 15.

679 Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates
680 of *Fusarium oxysporum* f . sp . *ciceris*. Archives of Phytopathology and Plant Protection
681 43(2): 174–190.

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

684 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

708 Applied Research on Medicinal and Aromatic Plants 100244.
709 Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New
710 York.
711 O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the
712 fungus causing Panama disease of banana : Concordant evidence from nuclear and
713 mitochondrial gene genealogies. Proc. Natl. Sci. USA 95: 2044–2049.
714 Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt , focusing on bananas.
715 Appl Soil Ecol 132: 20–33.
716 Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium
717 species isolated from weeds and plant debris in Croatia. J Phytopathol 160: 76–81.
718 Statistics Indonesia. (2018). Timber Culture Establishment. BPS-Statistics Indonesia, Jakarta.
719 Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal
720 agent of mung bean wilt in China. Crop Protection 117: 77–85.
721 Suwandi, Akino S, Kondo N (2012) Common spear rot of oil palm in Indonesia. Plant Dis 96(4):
722 537–543.
723 Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez
724 JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant
725 Pathol 40(2): 238–247.
726 Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum* fungus: A
727 cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2): 110–118.
728
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730

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

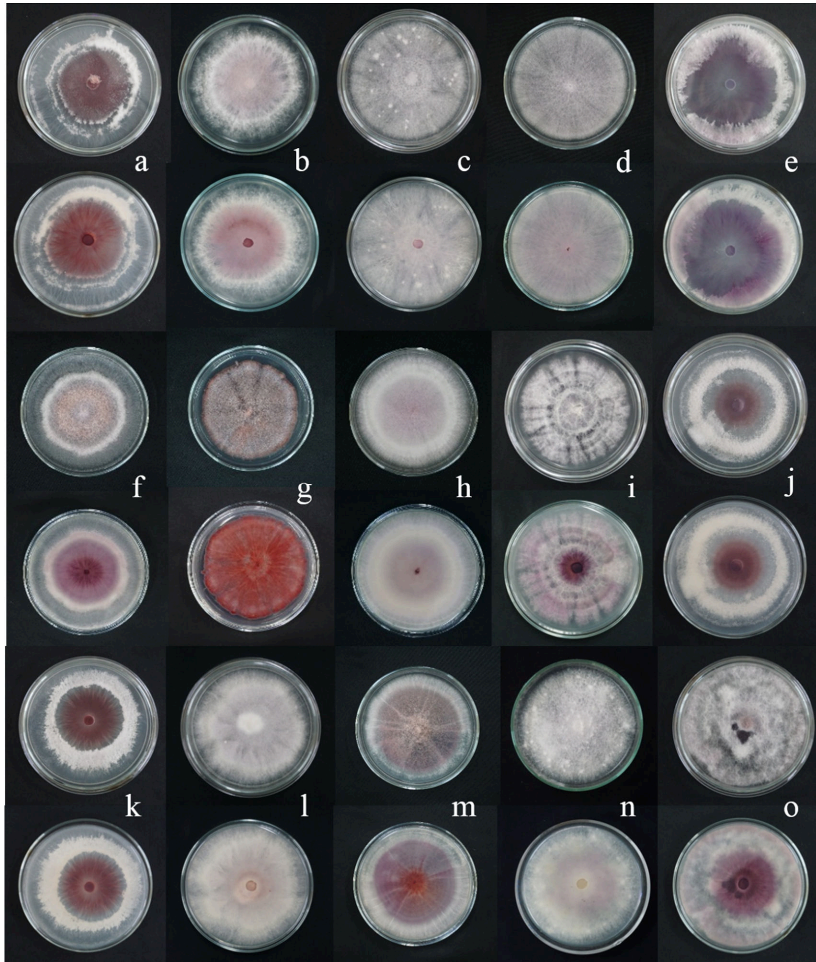
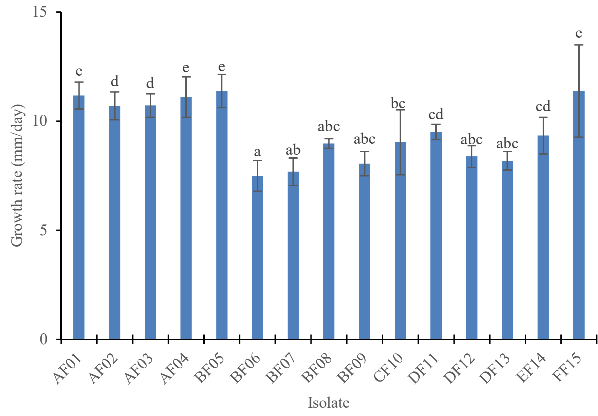


Fig. 2 Colony character on PDA: AF01(a), AF02 (b), AF03 (c), AF04 (d), BF05 (e), BF06 (f), BF07 (g), BF08 (h), BF09 (i), CF10 (j), DF11 (k), DF12 (l), DF13 (m), EF14 (n), and FF15 (o). Colony on top surface (1st, 3rd, and 5th line), Colony on bottom surface (2nd, 4th, and 6th line).

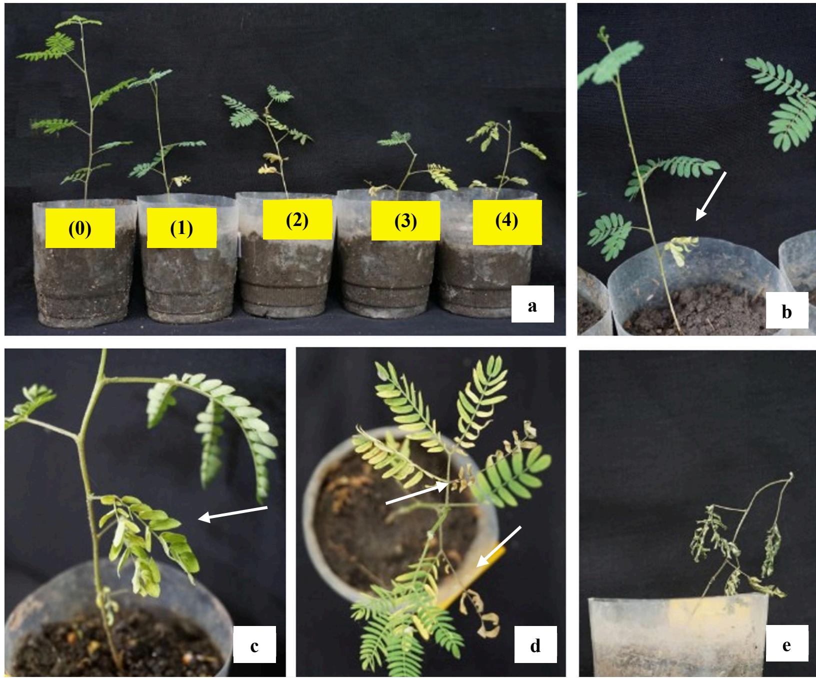
736



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

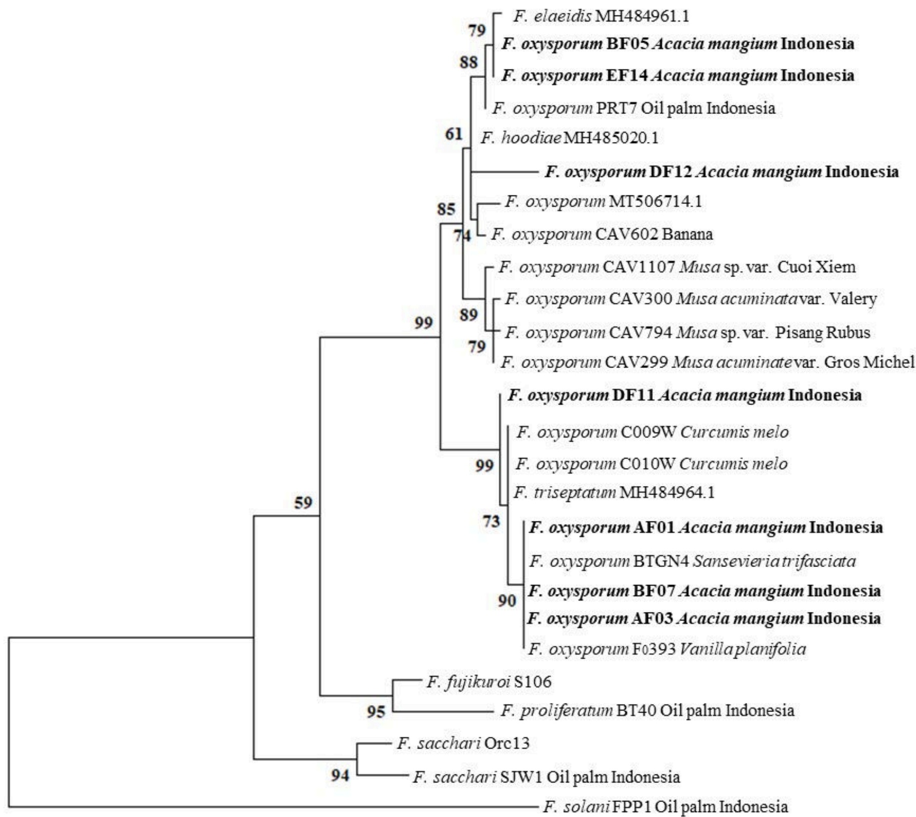
738

739



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

743



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

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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

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

756

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

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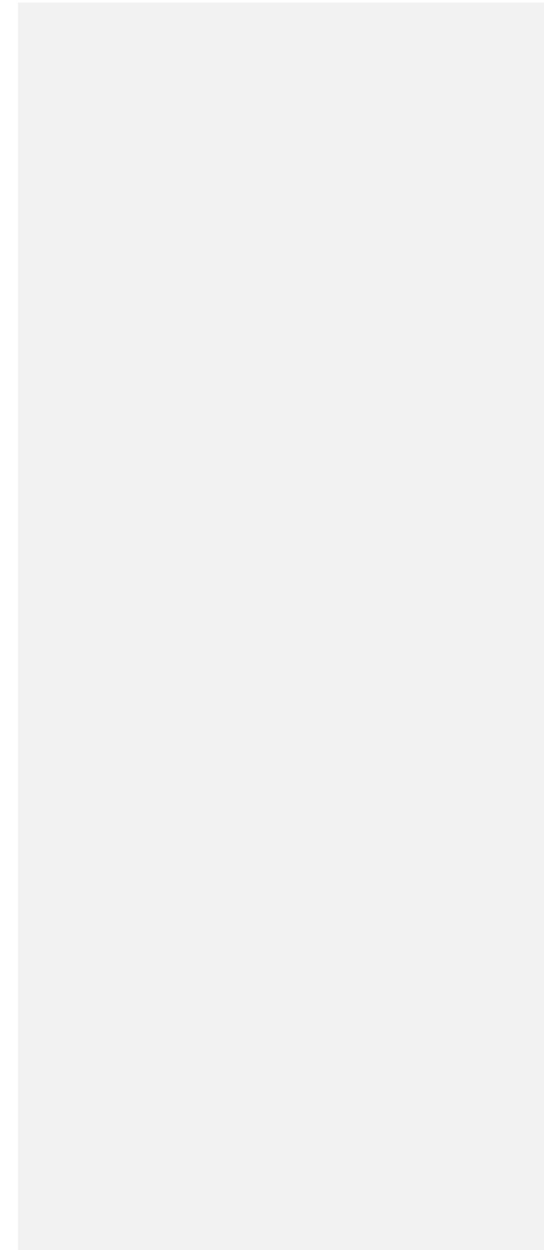

759 Table 4 Incidence, severity and progression of wilt disease in one-month-old acacia

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}~~a~~	46.4 a
AF03	80	1.9 ^{ab}~~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

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

762 in the LSD test at 5%~~.~~

763

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PERBAIKAN PROOFREAD

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

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~~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*.

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

417

418 **Introduction~~NTRODUCTION~~**

419 Black wattle (*Acacia mangium* Willd.) is a flowering~~green leafy plant~~tree species native to Papua, 420 West Irian Jaya and Maluku in Indonesia, Papua New Guinea and northeast Queensland in 421 Australia. Under favorable ~~environmental~~ conditions, this species can grow~~,~~ up to 30 m with a 422 diameter of 50 cm. It is cultivated on industrial ~~forest~~ plantations by large companies for its~~because~~ 423 ~~it gives~~ high levels of ~~good~~ quality pulp and a good paper yield (Hedge et al. 2013). In 2018, the 424 total land area controlled by industrial plantation forest companies in Indonesia was~~ere~~ 8.67 425 million hectares, with 81.3~~0~~% ~~being~~ used for plant cultivation, including *A. mangium*. 426 ~~Furthermore,~~ In addition, in 2017, this ~~plant~~species produced the most logs of any other species, 427 reaching 77.5~~5~~% from a log total of 40,628.8~~78~~ m^3 (Statistics Indonesia 2018).

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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.

428 ~~The~~A major~~in~~ problem ~~faced~~ in its cultivation is an unknown cause of seedling wilt disease. 429 It occurs during the initial stages of ~~plant~~ growth and~~,~~ if ignored, ~~it~~ can spread widely in a 430 plantation.

431 *Fusarium oxysporum* ~~*F. oxysporum*~~ Snyder & Hansen is a soil-borne pathogen with a ~~very~~ 432 wide ~~host~~ range of hosts and is common in various regions of the world (Bayona et al. 2011; Orr 433 and Nelson 2018; Edel-Hermann and Lecomte 2019), including forests and industrial plantations 434 (Widyastuti et al. 2013). This pathogen attacks all phases of growth (vegetative and regenerative) 435 and ~~is able to~~ survives on plant debris for ~~a~~ long periods (Postic et al. 2012; Meena and Roy 2020). 436 It causes vascular wilt or root rot disease in plants (Bayona et al. 2011; Gordon 2017). The 437 symptoms involve chlorosis ~~in~~of the leaves, stunted growth, discoloration of the plant's vascular 438 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.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 Ggreenhouse House of the Forest Research Institute, India (Kapoor et al. 2004). On *A. mangium* iIn Papua (Indonesia), *F. oxysporum* has been found to cause damping-off disease inon *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 nurseriesy 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 driedy up. The symptoms started from the lower leaves, moving on to the upper leaves and the shoots. Plant sSamples showing wilting symptoms were collected and stored in a cool box. during the process. To determine soil infectivity, acacia seeds were sown usingon 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 radicule development. The germinated seeds were planted on infested field nursery

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462 soil.~~medium.~~ The infected seedlings from the field and the infe~~cted~~sted ~~soil~~ nursery soil ~~medium~~

463 were sampled and the pathogen ~~was also~~ isolated from the plant tissue.

464

465 **Fungal isolation**

466 Fungi were isolated from the roots of plants growing in the field that showed ~~wilt~~ symptoms of

467 wilt and ~~also~~ from soil infe~~cted~~sted with pathogens. ~~Then, t~~The root samples were then washed

468 under running water~~,~~ and the surface ~~was~~ sterilized by dipping ~~them~~ in a solution containing 1%

469 sodium hypochlorite for 2 min, rinsing ~~them~~ three times in~~using sterile~~ distilled water, and drying

470 ~~them~~ on filter paper in a laminar airflow (Suwandi et al. 2012). ~~Then, t~~They were then laid out on

471 ~~planted in~~ a Petri plate of~~containing agar water with~~ 2% (w/v) agar and 0.1% ~~S~~streptomycin~~-~~

472 sulfate, and incubated for 48 h (Gardner 1980; Leslie and Summerell 2006; Suwandi et al. 2012).

473 The mycelium ~~that grew~~ from the root cuttings was transferred to potato dextrose agar (PDA)

474 ~~(Merck, Germany)~~ medium using the single hyphae method. The isolate results were used for

475 further research.

476

477 **Morphological identification**

478 The initial identification was carried out based on ~~the~~ Leslie and Summerell (2006). ~~method.~~ The

479 observation of cultural characteristics and the morphology of the colony included growth rates and

480 color pigments produced on PDA media. Asexual spores and other structures were observed ~~based~~

481 on the growth produced on carnation leaf agar (CLA) medium. These were made under a light

482 microscope (OLYMPUS CX 23) at 1000 × magnification with a camera (Optilab Advance Plus,

483 Yogyakarta, Indonesia). Measurements were carried out on 100 spores of microconidia~~,~~ and

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484 macroconidia, and chlamydospores using Image Raster 3.0 software with magnification adjusted

485 to a microscope.

486

487 **Pathogenicity test**

488 ~~A pathogenicity~~This test was carried out on *A. mangium* seedlings 30 days after sowing~~. This plant~~

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489 ~~was grown~~ in plastic pots containing 200 g peat soil ~~medium (200 g)~~ which had previously been

490 sterilized. ~~using an autoclave.~~ Fungal isolates were grown ~~i~~on a potato dextrose broth (PDB)

491 medium by placing 5 × 5 mm agar pieces of the fungal colony in the broth. The cultures were

492 incubated for three days using a shaker at a speed of 120 rpm to produce large quantities of conidia.

493 This suspension was used as inoculum by pouring 1×10^6 cfu g^{-1} (colony forming unit/g) ~~soil~~ ~~i~~on

494 a soil medium~~,~~ while the uninoculated control was ~~watered only with~~ sterile distilled water. Each

495 isolate was inoculated into the soil of 10 test plants and the experiment ~~was~~ repeated once. Disease

496 incidence was counted ~~by~~as the number of diseased plants out of ~~the~~ 10. ~~tested plants.~~ The severity

497 of the disease was calculated for each seedling using a score of 0–4, where 0 = no disease/healthy

498 seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead

499 seedling. The plants were ~~then~~ observed ~~for 1–~~over 30 days after inoculation. The difference in

500 disease severity and the area under the disease progress curve (AUDPC) between isolates was

501 calculated through ANOVA and Tukey's HSD test. An analysis was performed using the SAS

502 university edition software package.

503

504 **Molecular identification**

505 Seven representative~~s~~ fungal isolates with distinct morphological characteristics were selected and

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506 grown ~~in a liquid medium of PDB~~ in a cultivation bottle containing 50 mL sterile PDB (200 g

507 potato; 20 g glucose; 1 L distilled water). ~~s~~Sections of 3–4- day-old cultures on the PDA medium,

508 measuring 5 × 5 mm, were placed ~~i~~on a PDB liquid medium and incubated for 3–4 additional~~more~~

509 days at room temperature. ~~Meanwhile, t~~The fungal mycelium was harvested using vacuum

510 filtration and ~~then it was~~ frozen. ~~The~~ DNA was extracted using the YeaStar Genomic DNA Kit

511 (Zymo Research Corporation, Irvine, CA,~~California,~~ USA) following manufacturer's instructions.

512 Its concentration and quality were determined by spectrophotometry using a NanoDrop

513 Spectrophotometry ND‑1000 (NanoDrop Technologies, Montchanin, DE,~~elaware,~~ U.S.A.) and

514 stored at -20 °C until used. The translation elongation factor 1-α (*tef1*) was amplified using primers

515 EF1 (forward: 5′ -ATGGGTAAGGAAGACAAGAC -3′) and EF2 (reverse:

516 5′-GGAAGTACCAGTGATCATGTT-3′) (O'Donnell et al. 1998). PCR was carried out in 50 µL

517 of the reaction mixture containing 20 µL Master Mix (Eppendorf, Germany) (1.25 GoTaq DNA

518 polymerase, 0.2 µM of each dNTP, 2 × PCR buffer), 1 µL of each primer, and 2 µL of DNA

519 template. The amplification was performed using a PCR Cycler Termal C1000 Touch ™ (Bio-

520 rad, USA). The initial denaturation was performed for 2 min at 95 °C followed by 30 cycles for

521 20 s at 95 °C, annealing for 40 s at 58 °C, and extension for 1 min at 65 °C, with a final elongation

522 step of 5 min at 65 °C (Suwandi et al. 2012). Sequences of the PCR products were analyzed at 1st

523 BASE, Co., Ltd., Kuala Lumpur, Malaysia. The sequence data obtained was determined by

524 comparing the GenBank (http://www.ncbi.nlm.nih.gov) and FUSARIUM-ID databases. To

525 determine the genetic relatedness of *Fusarium oxysporum* from *A. mangium* with the known

526 *Fusarium* population, the *tef1* sequences were aligned using Clustal-W in MEGA7 and maximum

527 parsimony (MP) analyses were performed. There was a total of 577 positions in the final dataset.

528 All positions containing gaps and missing data were eliminated. The MP tree was obtained using

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

530 2016).

531

532 **Results ESULTS AND DISCUSSION**

533 **Results**

534 ***Disease symptoms and wilt incidences***

535 Surveys on seedling wilt were carried out in six acacia nurseriesy locations inof commercial

536 companies;, with five sites in the Air Sugihan area and one site in Lebong Hitam (Table 1). The

537 incidence of disease varied between locations from as low as 6.0% up to 36.9%. The early

538 symptoms of seedling wilt started fromas the lower leaves as they turned yellow,, and then black,

539 dried out, fell and the plant died. Some symptoms began with yellowish discoloration of the leaves,

540 which then wilted, and some had no discoloration but immediately began with wilting (Fig. 1).

541 Observations were made by planting acacia seedlings on used soil medium from the commercial

542 nursery, and the results showed that 56.255% of the seedlings were attacked. In the commercial

543 nurseries, y, this disease was found to attacks acacia seedlings at an average age of more than 1

544 month after germination and before the formation of phyllodes or modified petioles or stems.false

545 leaves.

546

547 ***Morphological characteristics***

548 Fifteen isolates similar to *F. oxysporum* were takenisolated from the root tissues of diseased plants

549 (Table 2), and grown on PDA and CLA media (water agar medium with carnation leaf pieces) at

550 a temperature of 27 °C. The PDA cultures on the PDA medium produced several color pigments,

551 such as purple, pale purple and reddish-pink pigments, with air hyphae (Fig. 2). These isolates

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552 showed varied growth rates of the colony, with the fastest on BF05 and FF15 isolates (11.4~~38~~
553 mm/day). BF06 had~~showed~~ the slowest ~~colony~~ growth rate (7.5~~46~~ mm/day) ~~compared to the others~~
554 (Fig. 3). They all produced ~~many~~numerous microconidia on the false heads of monophialides.
555 Hyaline microconidia are oval, elliptical, reniform to allantoid with an average size of 5.5 ± 1.0
556 µm × 2.8 ± 0.5 µm, to 11.8 ± 4.1µm × 3.0 ± 0.3 µm, and have zero~~0~~ to ~~1~~one septum~~,~~ but generally
557 zero.~~0.~~ The hyaline macroconidia, being sickle-shaped, have an average size of 33.6 ± 5.4 × 3.9 ±
558 0.5 µm, to 39.7 ± 5.9 × 3.8 ± 0.4 µm, and have three~~3~~ to seven~~7~~ septa~~,~~ but usually~~generally~~ ~~3~~three.
559 The chlamydospores produced singly or~~/~~ in pairs at the terminal/intercalary have an average
560 diameter of 6.4 ± 0.6 µm to 10.0 ± 2.5 µm (Table 3). Based on the morphological characteristics
561 of the PDA and CLA media, all isolates were in accordance with the *F. oxysporum* as described
562 by Leslie and Summerell (2006).
563
564 ***Molecular characteristics***
565 ~~The m~~Molecular identification ~~achieved~~ by sequencing the *tef1* gene was amplified using primers
566 EF1 and EF2 against seven selected isolates. The sequence data was determined by ~~making~~ a
567 comparison with those ~~existing~~ in Genbank through the BLAST and FUSARIUM-ID programs.
568 Based on this, the sequences were confirmed as *F. oxysporum* with 84.9−100% similarity to
569 Genbank and 97.3–99.7% with the FUSARIUM-ID. Phylogenetic analysis (consistency index 0.8,
570 retention index 0.9 and the composite index 0.8) grouped all *Fusarium* isolates within the *F.*
571 *oxysporum* species complex. Isolates were clustered within four clades (Fig. 5). The first clade
572 comprised~~nsisted of~~ BF05 and EF14, and *F. elaeidis* (MH484961.1) from *Elaeis* spp. The second
573 clade consisted of single isolates from *A. mangium* (DF11), *F. oxysporum* (C009W and C010W)
574 from *Cucumis melo*, and *F. triseptatum* (MH484964.1) from *Ipomoea batatas*. The third clade

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575 contained~~consisted of~~ AF01, AF03 and BF07 along with BTGN4 (*F. oxysporum* from *Sanseviera*
576 *trifasciata*) and FO393 (*F. oxysporum* from *Vanilla planifolia*). The fourth included~~consisted 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~~ ~~fell~~falling from the plant. This
586 ~~symptom~~ progresses to the top of the plant, causing it to wither and die. ~~Furthermore~~In 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 showed~~experienced~~ 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 to~~Concerning~~ disease incidence, the *Fusarium* isolates
595 had~~presented~~ a high variation in percentage of disease incidence, ranging from 50−100%. These
596 isolates also induced wilting severities of~~ranging 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

598 (3.2). Based on disease severity, the isolates were grouped into three categories of virulence: high
599 (score 2.0−4.0), moderate (score 1.2−2.0), and low score (0−1.2) virulence (Dubey et al. 2010).
600 BF08, AF01, AF02, AF04, CF10, DF12, DF13, BF06, and DF11 were included in the high
601 virulence group (2.1−3.2), while FF15, BF09, BF07, AF03, and BF05 were in the moderate (1.6−
602 2.0), and only EF14 had a~~was in the~~ low score. We observed that this pathogen causes vascular
603 disease, where the pathogen could be isolated from the basal stem up to the shoots. To confirm the
604 Koch's Postulates test, the pathogens were all re-isolated (100%) from infected plants and it was
605 confirmed that the morphological characters of the pathogen were the same as the inoculated
606 *Fusarium* isolates. ~~-~~The pathogen was not isolated from the uninoculated control plants.
607
608 **Discussion**
609 This research reports that ~~*F*~~ *Fusarium*~~.~~ *oxysporum* ~~was~~has been identified for the first time
610 as a causative agent for *A. mangium* seedling wilt in South Sumatra, Indonesia. According to
611 previous studies, *F. oxysporum* ~~has been~~was reported ~~to be~~as the cause of seedling wilt in *A. koa*
612 in Hawaii (Gardner 1980) and *A. nilotica* in India (Kapoor et al. 2004). In Indonesia, this pathogen
613 was reported to attack ~~acacia~~ germinated acacia seeds, resulting in damping-off, and the
614 identification of this was made solely ~~based~~ on morphological characters (Widyastuti et al. 2013).
615 Our~~This~~ research confirms that *F. oxysporum*, identified by morphological and molecular
616 methods, causes vascular wilt disease in advanced seedling stages. Another study by Luo and Yu
617 (2020) has demonstrated that *F. oxysporum* causes damping-off on *Pinus massoniana* Lamb.
618 The pathogenicity test confirmed that all the isolates were able to develop wilt symptoms
619 with different disease incidence and severity, some reaching 90−100% and scoring 2.1−3.2,
620 respectively. Other studies have reported that *F. oxysporum* causes wilt diseases on *A. nilotica* in

621 India with diseases severity of 16.9~~86~~% (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).

624 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.

629 *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).

638 ~~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 infec~~s~~ted 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

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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 infe~~c~~sted 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 ~~present~~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 ~~a~~seedling soils ~~seedling medium~~ infe~~c~~sted 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.

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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".

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662 ~~REFERENCES~~

663 References

664 Altinok HH (2013) Fusarium species isolated from common weeds in eggplant fields and
665 symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. J Phytopathol 161:
666 335–340.

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

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

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

675 Dobbs JT, Kim MS, Dudley NS, Klopfenstein NB, Yeh A, Hauff RD, Stewart JE (2020) Whole
676 genome analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) and the
677 development of molecular tools for early detection and monitoring. BMC Genomics 21: 1–
678 15.

679 Dubey SC, Singh SR, Singh B (2010) Morphological and pathogenic variability of Indian isolates
680 of *Fusarium oxysporum* f . sp . *ciceris*. Archives of Phytopathology and Plant Protection
681 43(2): 174–190.

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

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

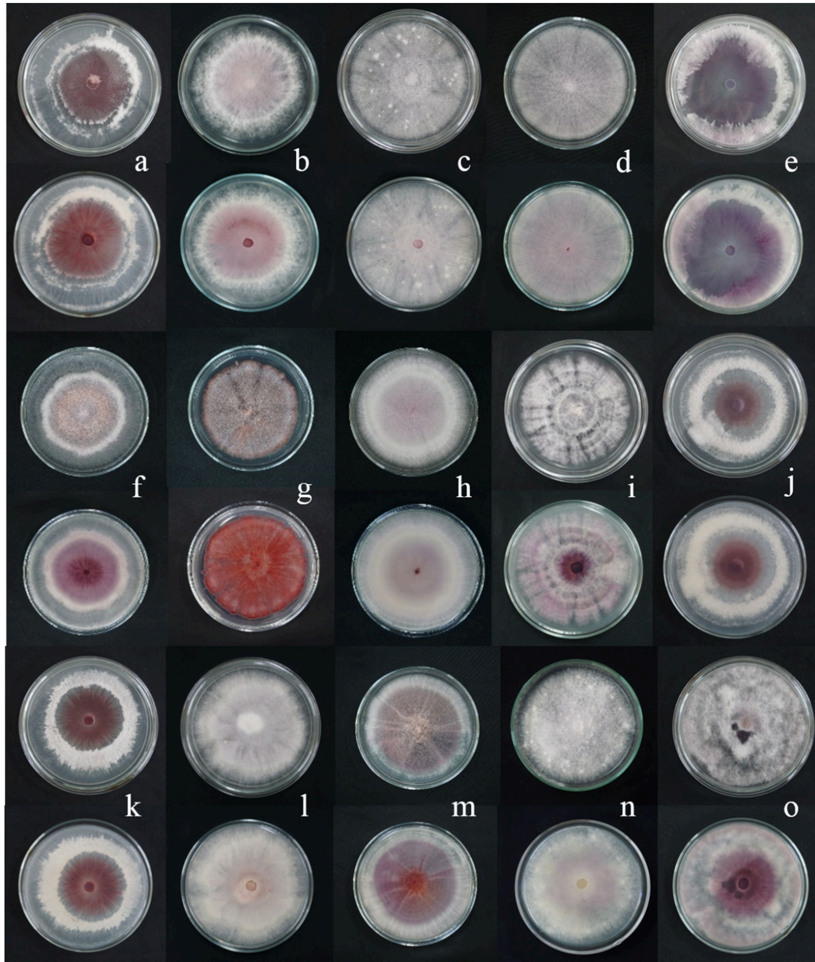
685 nov. J Phytopathol 70(7): 594–597.
686 Gordon TR (2017) *Fusarium oxysporum* and the Fusarium Wilt Syndrome. Annu Rev Phytopathol
687 55: 23–39.
688 Hedge M, Palanisamy K, Yi JS (2013) *Acacia mangium* Willd . A Fast Growing Tree for Tropical
689 Plantations. J For Sci 29(1): 1–14.
690 Jiménez-Díaz 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

708 Applied Research on Medicinal and Aromatic Plants 100244.
709 Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New
710 York.
711 O'Donnell K, Kistler HC, Cigernik E, Ploetz RC (1998) Multiple evolutionary origins of the
712 fungus causing Panama disease of banana : Concordant evidence from nuclear and
713 mitochondrial gene genealogies. Proc. Natl. Sci. USA 95: 2044–2049.
714 Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt , focusing on bananas.
715 Appl Soil Ecol 132: 20–33.
716 Postic J, Cosic J, Vrandecic K, Jurkovic D, Saleh AA, Leslie JF (2012) Diversity of Fusarium
717 species isolated from weeds and plant debris in Croatia. J Phytopathol 160: 76–81.
718 Statistics Indonesia. (2018). Timber Culture Establishment. BPS-Statistics Indonesia, Jakarta.
719 Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal
720 agent of mung bean wilt in China. Crop Protection 117: 77–85.
721 Suwandi, Akino S, Kondo N (2012) Common spear rot of oil palm in Indonesia. Plant Dis 96(4):
722 537–543.
723 Velarde-Félix S, Garzón-Tiznado JA, Hernández-Verdugo S, López-Orona CA, Retes-Manjarrez
724 JE (2018) Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can J Plant
725 Pathol 40(2): 238–247.
726 Widyastuti SM, Tasik S, Harjono (2013) Infection process of *Fusarium oxysporum* fungus: A
727 cause of damping-off on *Acacia mangium*'s seedlings. Agrivita 35(2): 110–118.
728
729

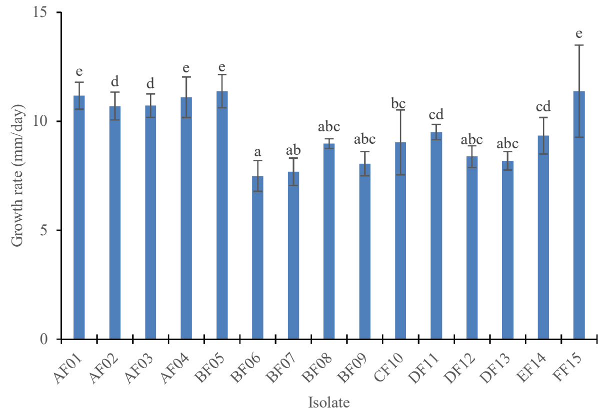
730

731 Fig. 1 (a) Wwilt symptoms in the nursery, (ab) yellowing leaves, (bc) dry leaves (c).

732



733 Fig. 2 Colony character on PDA: (a) AF01~~(a)~~, (b)AF02 ~~(b)~~, (c) AF03 ~~(c)~~, (d) AF04 ~~(d)~~, (e) BF05
734 ~~(e)~~, (f) BF06 ~~(f)~~, (g) BF07 ~~(g)~~, (h) BF08 ~~(h)~~, (i) BF09 ~~(i)~~, (j) CF10 ~~(j)~~, (k) DF11 ~~(k)~~, (l) DF12 ~~(l)~~,
735 (m) DF13 ~~(m)~~, (n) EF14 ~~(n)~~, and (o) FF15 ~~(o)~~. (1st, 3rd, and 5th line) Colony on top surface ~~(1st, 3rd,~~
736 ~~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

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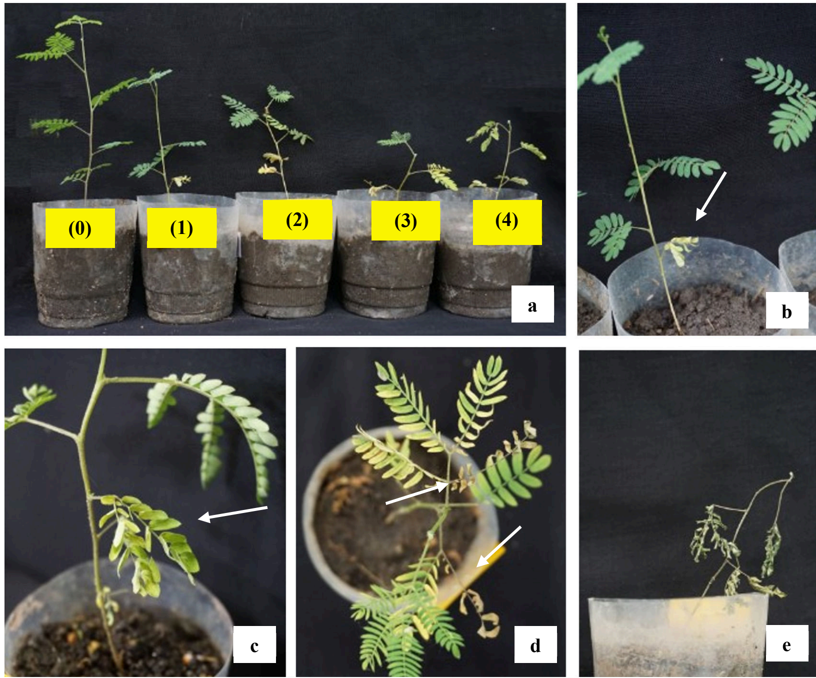
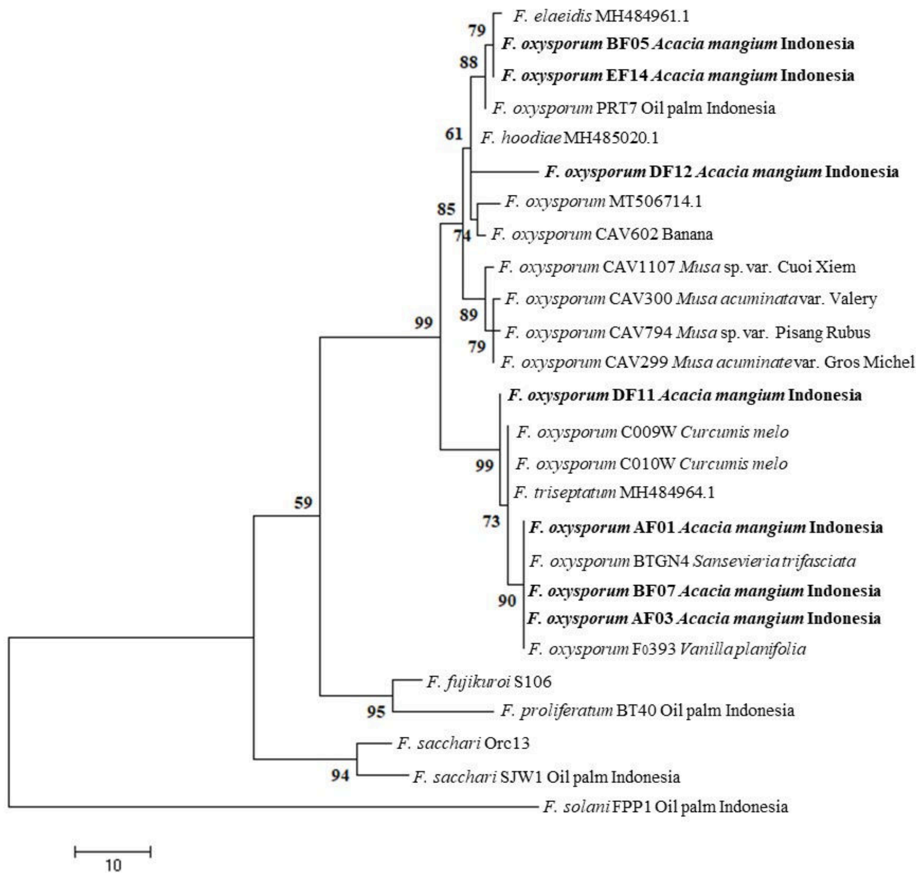


741 Fig. 4 Disease severity rate:~~.~~ (a) ~~F~~from left: healthy plant to 100% wilted leaves (scale 0-4); ~~(a).~~
742 (b) ~~I~~initial symptoms: from lowest leaf, yellowing leaves; ~~(b).~~(c, d) ~~A~~advanced symptoms: curved
743 leaves, dry leaves, falling leaves; ~~(c,d). and~~(e) dead plant ~~(e).~~

744



745

746 Fig. 5 One out of the nine most parsimonious trees showing the genetic relatedness, represented
747 by the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium* (in bold), closely
748 related *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers,
749 host species and countries of origin are given with the representative isolates. A bootstrap test with
750 1000 replicates produced a bootstrap value greater than 50%, shown at the appropriate node. The
751 genetic distance is indicated by the scale bar.
752

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

756 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

758 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

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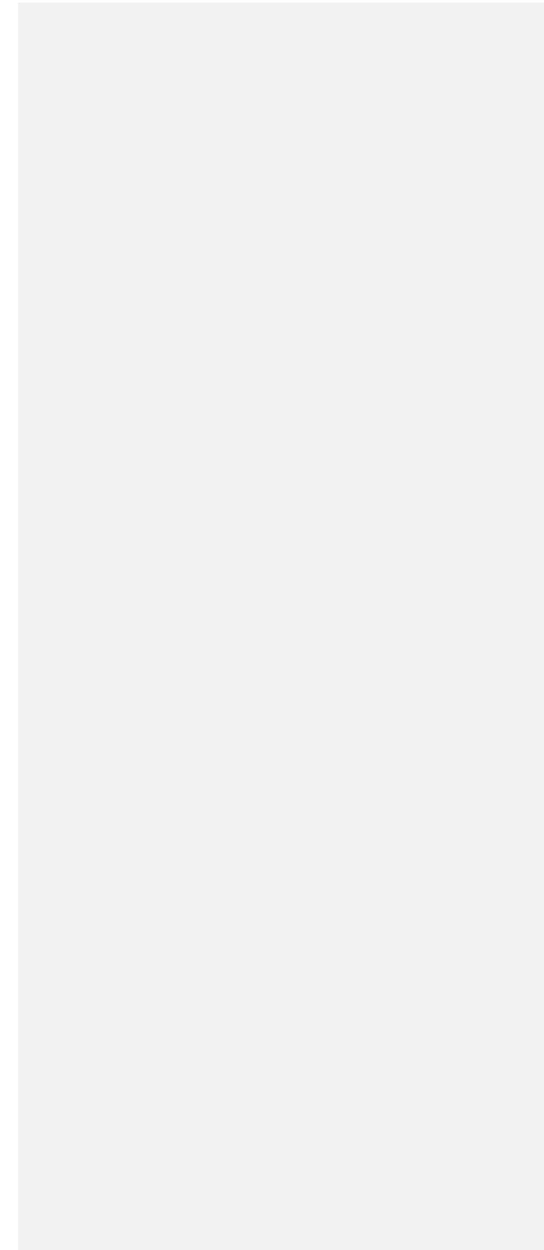

760 Table 4 Incidence, severity and progression of wilt disease in one-month-old acacia
761 seedlings inoculated with *F. oxysporum*

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

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

ORIGINAL PAPER

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

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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

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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

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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*

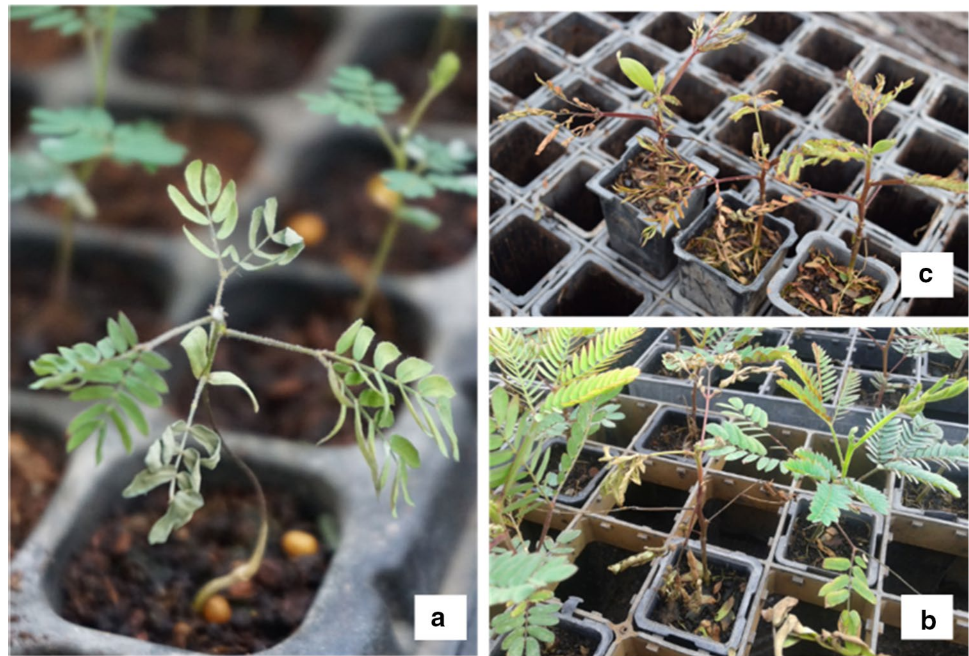

Fig. 1 **a** wilt symptoms in the nursery, **b** yellowing leaves, **c** dry leaves

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

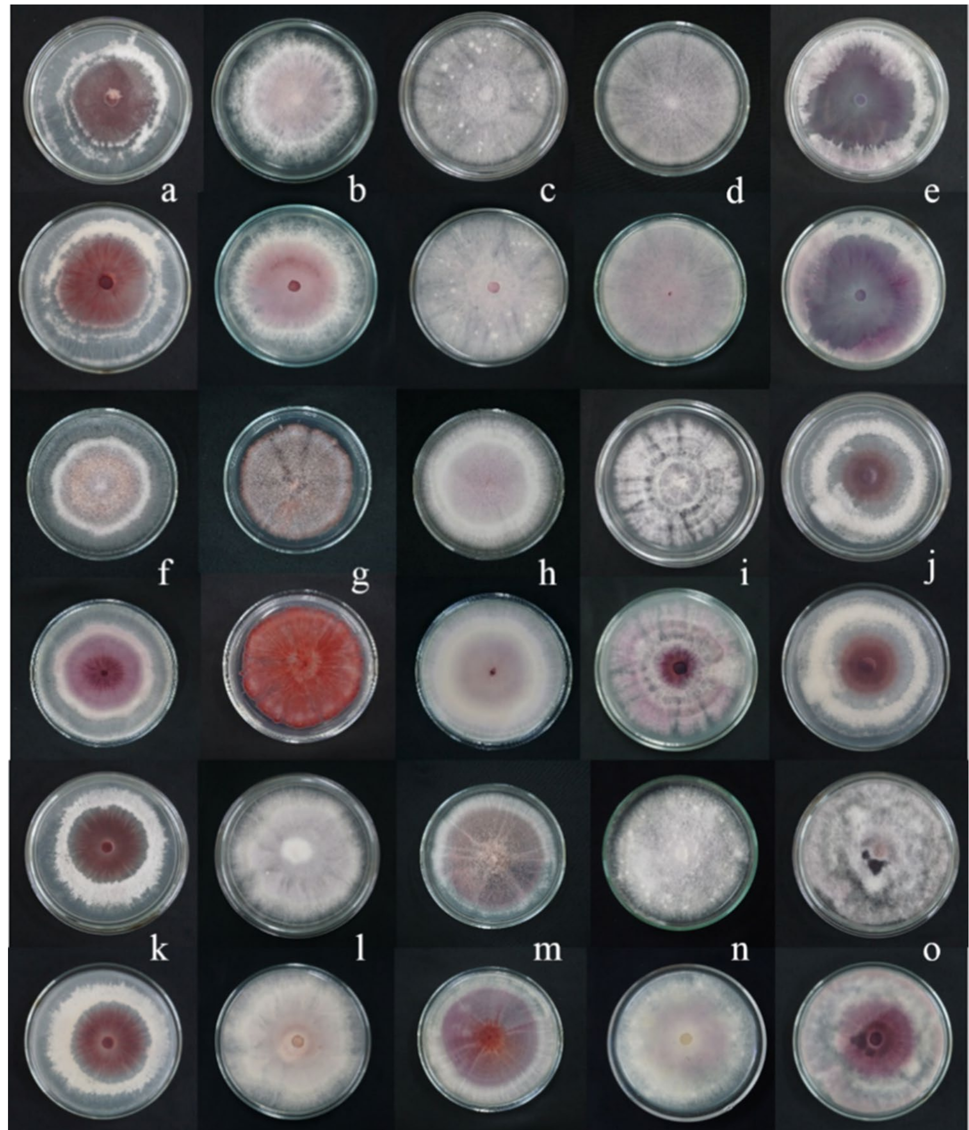

Fig. 2 Colony character on PDA; **a** AF01, **b** AF02, **c** AF03, **d** AF04, **e** BF05, **f** BF06, **g** BF07, **h** BF08, i BF09, **j** CF10, **k** DF11, **l** DF12, **m** DF13, **n** EF14, and **o** FF15. (1st, 3rd, and 5th line) Colony on top surface, (2nd, 4th, and 6th line) Colony on bottom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

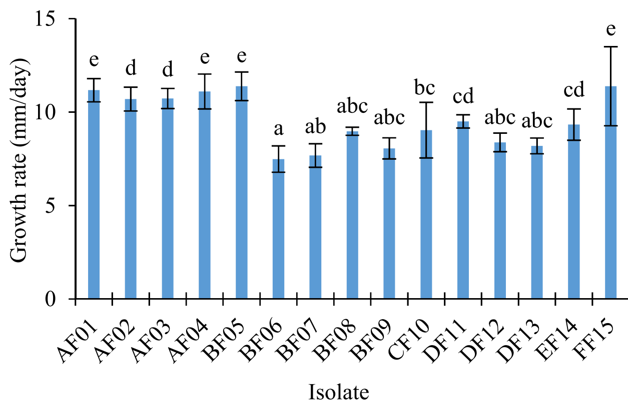

Fig. 3 Growth rate of *Fusarium oxysporum* from *Acacia mangium* on PDA medium

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

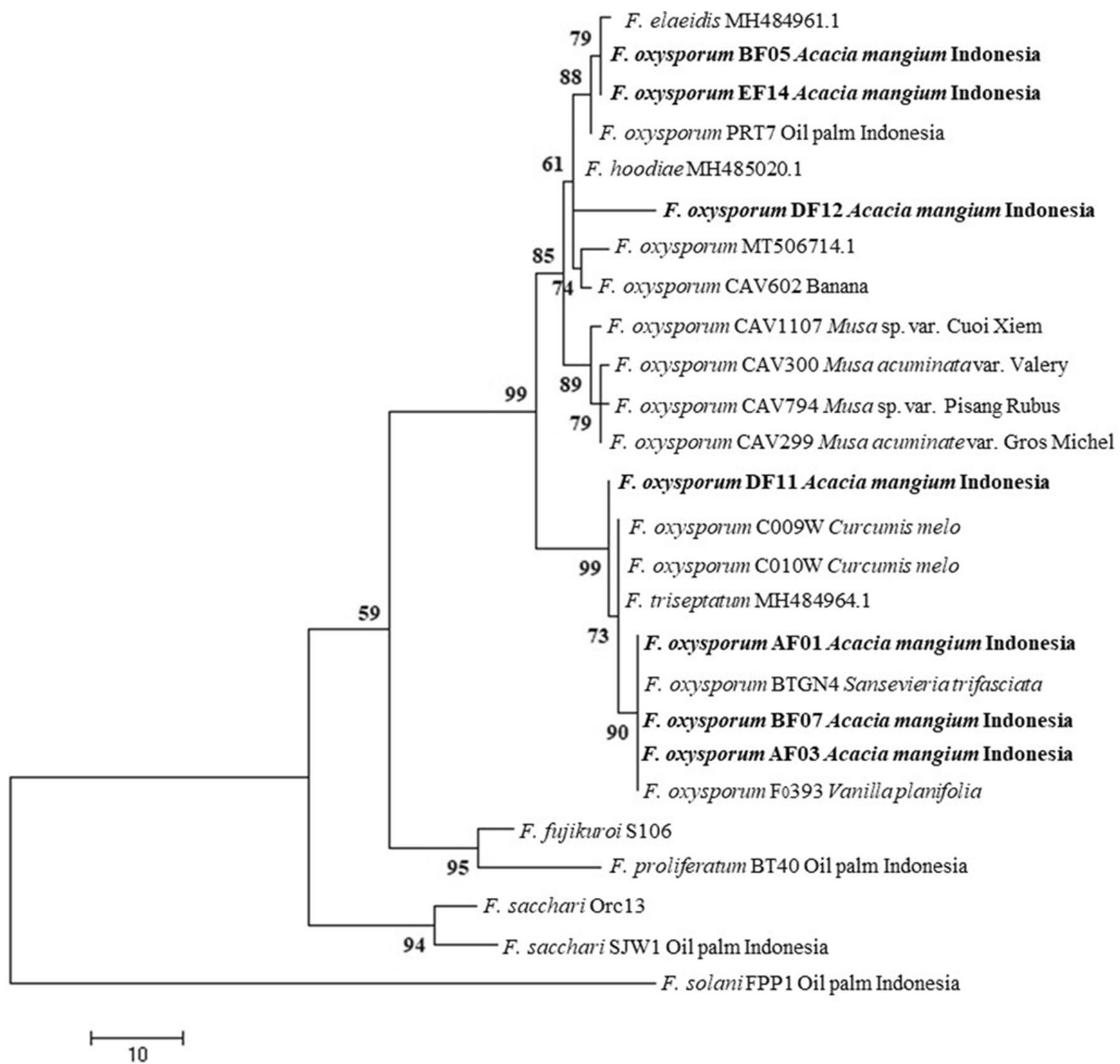

Fig. 4 One out of the nine most parsimonious trees showing the genetic relatedness, represented by the *tef1* sequences, between *Fusarium oxysporum* from *Acacia mangium*-(in bold), closely related *Fusarium oxysporum* isolates, and other species of *Fusarium* isolates. The strain numbers, host species 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

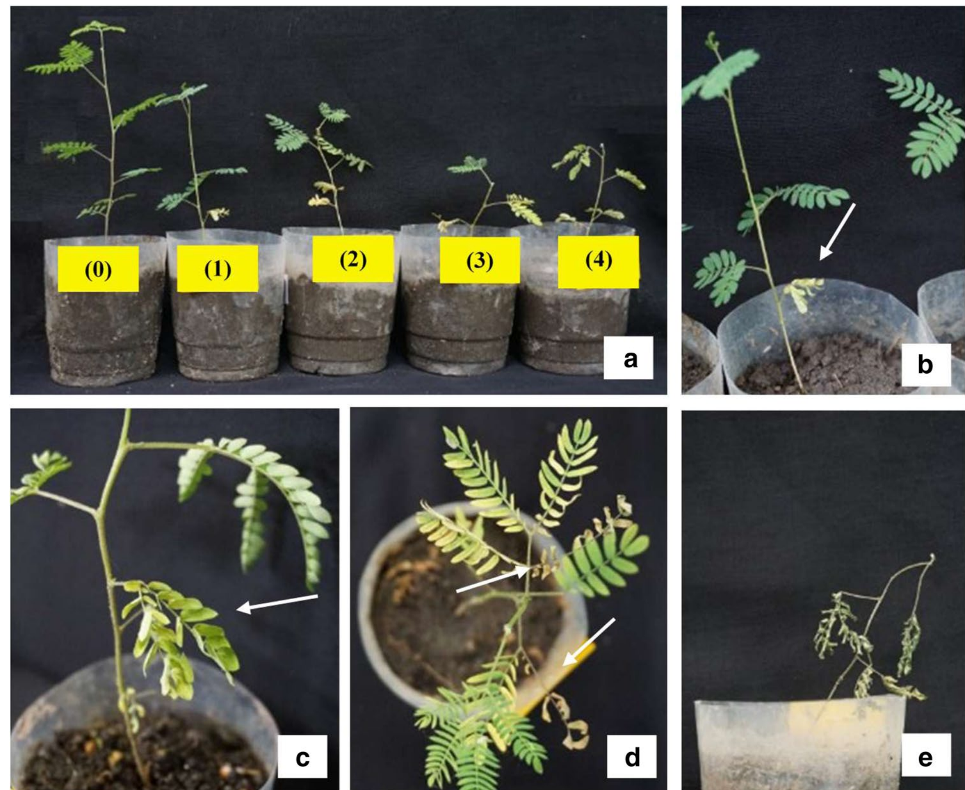

Fig. 5 Disease severity rate: **a** from left: healthy plant to 100% wilted leaves (scale 0 – 4); **b** initial symptoms: from lowest leaf, yellowing leaves; **c**, **d** advanced symptoms: curved leaves, dry leaves, falling leaves; **e** dead plant

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.

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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

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