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# Host Range Studies of *Fusarium oxysporum*, the Causal Agent of Seedling Wilt Disease of *Acacia mangium* Willd.

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**Abstract**

*Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra. It has been reported to have a high level of host specification at the genus or family level. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen on *A. mangium* and several forests and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-α) sequences were tested for pathogenicity on plants from the Fabaceae family and the growth of their population was observed. The results showed that it can infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and moderately resisted *by Acacia auriculiformis*. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while that of *L. leucocephala* was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, the *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the Fabaceae family.

**Keyword:** *Acacia mangium*, Fabaceae, *Fusarium oxysporum*, host range, seedling wilt.

## Introduction

*Acacia mangium* is a species of plant that originated in several regions in Indonesia, Papua New Guinea, and Australia, and which, for a few decades, has been introduced to the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It was planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asif et al. 2017), it is widely used in agroforestry, forestry, and for restoration of degraded land (Koutika and Richardson 2019).

*Fusarium oxysporum* is an important pathogenic fungus that causes wilt disease in different plants all over the world. Soleha et al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by this fungi are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006), more than 100 formae species and races have been identified and are widespread in the world.

Besides *A. mangium,* which is the main commodity of industrial forestry in Indonesia, other plants, such as, *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana,* and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum,* the causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen on *A. mangium* and several industrial and local forest plants in Indonesia.

## MATERIALS AND METHOD

### Fungal isolates

Three isolates of *F. oxysporum* (AF01, BF05, and DF11), which were differentiated according to their *tef* 1-α sequence, were utilised; they were chosen because a previous study (Soleha et al. 2021) had described them as the most pathogenic to *A. mangium* (Figure 1). Pathogen isolates were cultured in a PDB liquid medium (potato dextrose broth) and incubated at 26-28 °C on a shaker (150 rpm) for about five days. Then the mycelia suspension produced was filtered using two layers of sterile gauze to separate the conidia and hyphae. The conidia concentration was determined using a hemocytometer and then diluted to a concentration of $10^6$ $ml^{-1}$ for the pathogenicity test.

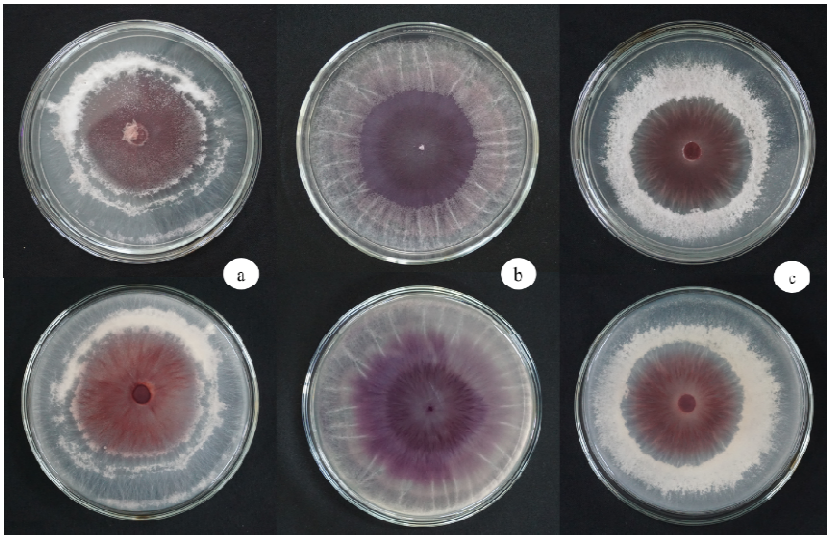


**Figure 1.** *F. oxysporum* isolates on PDA medium. (a) AF01, (b) BF05, and (c) DF11. First line: top surface; second line: bottom surface.

**Plant material**

The plants used were members of the Fabaceae (legumes) family, namely, *A. crassicarpa, A. auriculiformis*, *F. moluccana*, *A. pauciflorum*, *P. speciosa*, and *L. leucocephala*, which were one month old. The seedlings were obtained from the Forest Crops Research Institute, South Sumatra, transferred in a mixed medium with cocopeat (1:1) using a plastic pot that had a diameter and a height of 10 cm, and was placed in a shade house.

**Pathogenicity test**

A pathogenicity test was carried out using the root dip method (de Borba et al. 2017), where the roots were washed under running water and then immersed in 250 ml of conidia suspension ($10^6$ conidia $ml^{-1}$) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity was calculated using the method designed by Muslim et al. (2003a) and modified using a disease index (DI) 0-4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. Furthermore, plant responses were grouped as, R = resistant (D.I.=0), MR = moderately resistant/tolerance (DI = <1), MS = moderately susceptible (DI = 1.0-2.0), S = susceptible (DI = 2.1-3.0) and HS = highly susceptible (DI = 3.1-4.0) (Bertetti et al. 2018). The development of the disease was observed for 1–21 days after inoculation.

***Fusarium oxysporum* population**

The population of *F. oxysporum* in the roots was calculated at the end of the experiment using the method (Muslim et al. 2003b; Li et al. 2008; Horinouchi et al. 2011) with modifications to the surface sterilisation of the samples. Then the plants were grouped according to severity (disease score) and washed separately under running water to remove any soil residue adhering to the roots. After that, all plants in each score were surface sterilised using 1% sodium hypochlorite for 15 minutes, then rinsed three times with distilled water. The samples and water (1:100 w/v) were homogenised using a blender at a speed of 8000 rpm for ten minutes. Then they were filtered using two layers of sterile gauze and diluted 10 to 1000 times. The suspension was spread on Peptone PCNB agar media (Leslie and Summerell, 2006) in triplicate (five Petri dishes per replication) and incubated in the dark for seven days at room temperature. The number of colony-forming units (CFU) of *F. oxysporum* was calculated per gramme of sample's fresh weight and recorded based on the disease severity.

**RESULT**

**Pathogenicity test**

Three isolates of *F. oxysporum* were tested on six types of forest plants and the results showed that all plants tested had a similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e., yellowing of the oldest leaves closest to the stem base, which gradually progress to those younger and the shoots, severe wilting, drying, and falling of leaves, and lastly, death. Another symptom that appeared was the sudden wilting and death of the plant without the change of the leaves' colour; meanwhile, the control did not show any symptoms.

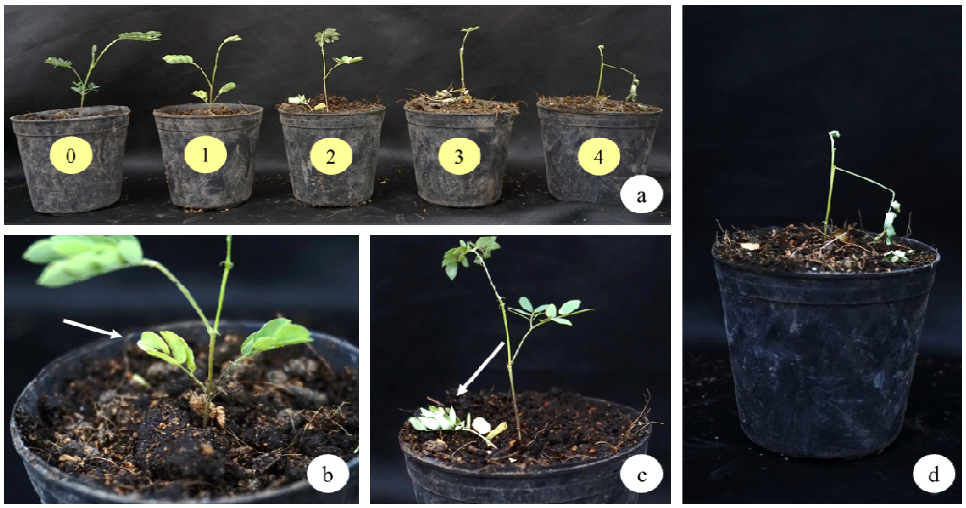


**Figure 2.** Disease index of *Acacia crassicarpa,* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant.

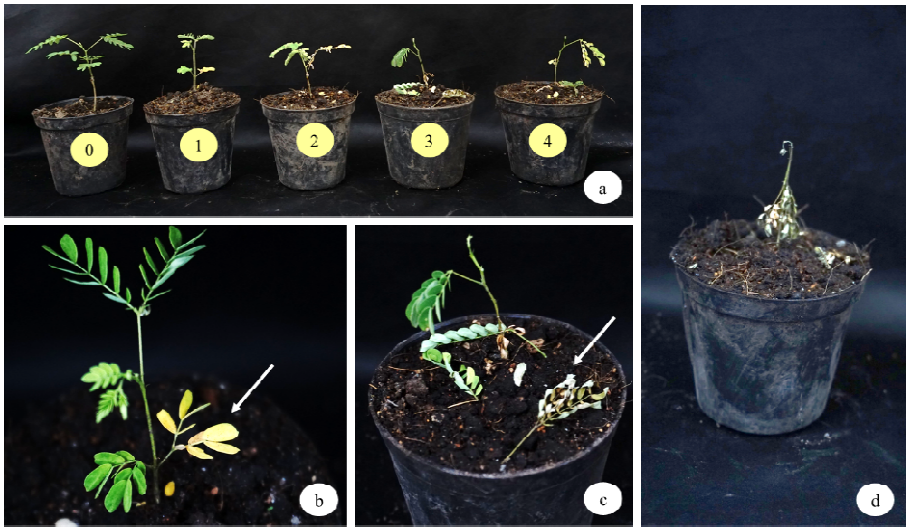


**Figure 3.** Disease index on *Falcataria moluccana* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant.

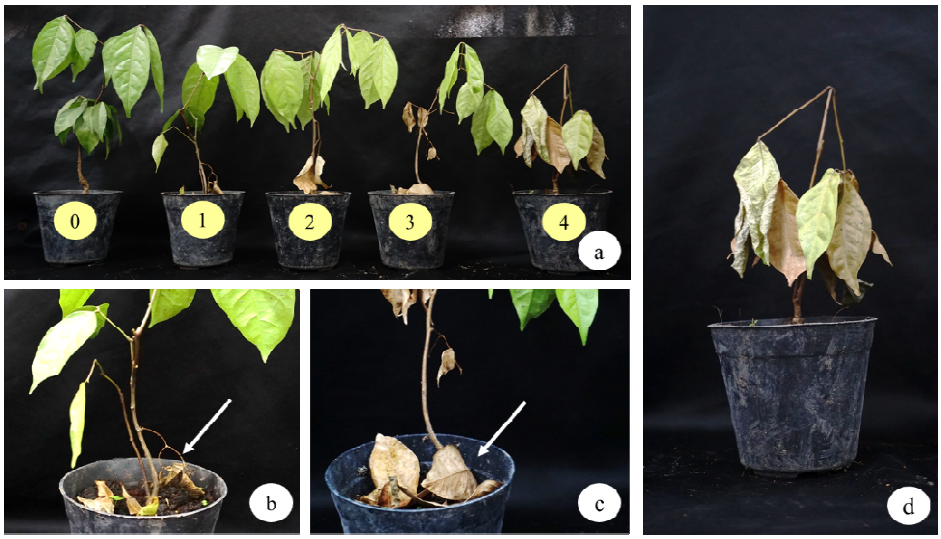


**Figure 4.** Disease index on *Archidendron pauciflorum,* (a) from left: healthy plant to 100% wilted leaves (score 0−4), (b) initial symptoms, yellowing and dry from oldest leaves, (c) advanced symptoms: falling leaves, (d) dead plant.

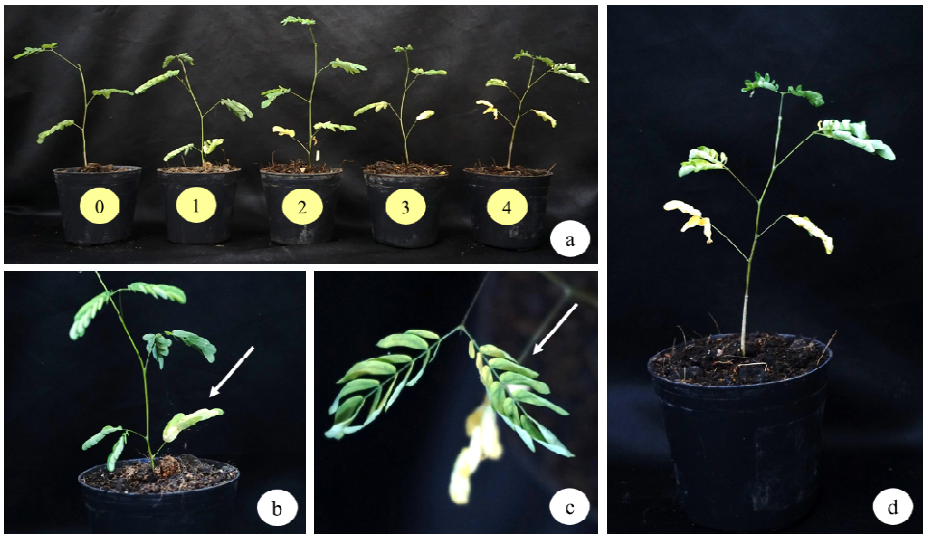


**Figure 5.** Diseases index on *Leucaena leucocephala,* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward.


**Figure 6.** Diseases index on *Parkia speciosa,* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; (d) dead plant.

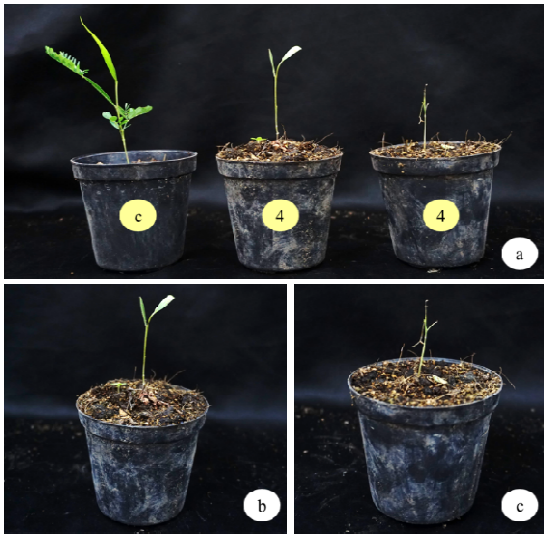


**Figure 7.** Diseases index on *Acacia auriculiformis*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b and c) advanced symptoms.

Disease severity caused by the inoculated *F. oxysporum* isolates was significantly higher than the controls. *A. crassicarpa* and *F. moluccana* were most severely affected with an average score of 4.00 and 3.44, and the incidence of wilting was 100%. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were attacked with moderate disease severity (scores 1.96, 1.68, and 1.80 respectively), and *A. auriculiformis* had the lowest severity and incidence (16% and 0.36 respectively) (Table 1). Based on the disease score on plant response, these are the three groups of responses by the hosts: i) highly susceptible (*A. crassicarpa* and *F. moluccana*), ii) moderately susceptible (*A. pauciflorum* *P. speciosa*, and *L. leucocephala*), and iii) moderate resistance/tolerance (*A. auriculiformis*) (Table 1).

Although the three isolates had different *tef1-α* genetic sequencing, they had similar virulence patterns. Therefore, there was no significant difference between the disease severity in the same host that was inoculated with different isolates.

**Table 1.** Pathogenicity and disease severity of *Fusarium oxysporum* isolated from *Acacia mangium*

| Plant species | Isolates [a)] | | | | | |
|---|---|---|---|---|---|---|
| | AF01 [b)] | Response [c)] | BF05 | Response | DF11 | Response |
| *Acacia crassicarpa* | 4.00 a | HS [c)] | 3.48 a | HS | 3.96 a | HS |
| *Falcataria moluccana* | 3.44 ab | HS | 3.04 a | HS | 2.80 ab | S |
| *Archidendron pauciflorum* | 1.96 bc | MS | 1.88 b | MS | 1.40 cd | MS |
| *Leucaena leucocephala* | 1.52 c | MS | 1.56 b | MS | 1.68 bc | MS |
| *Parkia speciosa* | 1.80 c | MS | 1.04 bc | MS | 2.16 bc | S |
| *Acacia auriculiformis* | 0.36 d | MR | 0.40 c | MR | 0.60 d | MR |

Values followed by the same letter in each row are not significant.
[a] DI 0-4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.
[b)] *F. oxysporum* isolates.
[c)] Host response were grouped as: R = resistant (D.I. = 0); MR = moderately resistant/tolerance (D.I. = <1); MS = moderately susceptible (D.I. = 1.0-2.0); S = susceptible (D.I. = 2.1-3.0); HS = highly susceptible (D.I. = 3.1-4.0) (Bertetti et al., 2018

***F. oxysporum* population**

The total population of *F. oxysporum* on the roots was determined by calculating the CFU for each category of damage. The results showed that at a score of 4, the three isolates which were tested on *A. crassicarpa* and *F. moluccana* showed a significantly higher population (82.00–105.10 × $10^4$ CFU $g^{-1}$ fresh weight) than the other plants. The lowest population was on *P. speciosa* and *A. pauciflorum* (3.57–12.27 × $10^4$ CFU $g^{-1}$ fresh weight). This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. auriculiformis.* In DI 1, the highest population was discovered in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample. Meanwhile, in asymptomatic plants (DI=0), the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was discovered in *A. crassicarpa* and *F. moluccana* (Table 2–Table 3).

The regression analysis results showed that all plants except *P. speciosa* had a linear relationship pattern between the increase in the disease score and the population. The pathogenic population on *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, as indicated by the magnitude of the regression gradient coefficient (m=20.3–21.3). However, the increase was moderate in *L. leucocephala* (m=11,2) (m=11.2) and very slow in *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* (m=2,2–4,8) (Figure. 8).

Table 3 shows that although the isolates were different in *tef1-α*, the population and DI patterns were similar for each test plant. The correlation between the population of pathogens ($g^{-1}$ fresh weight) and the level of DI are described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

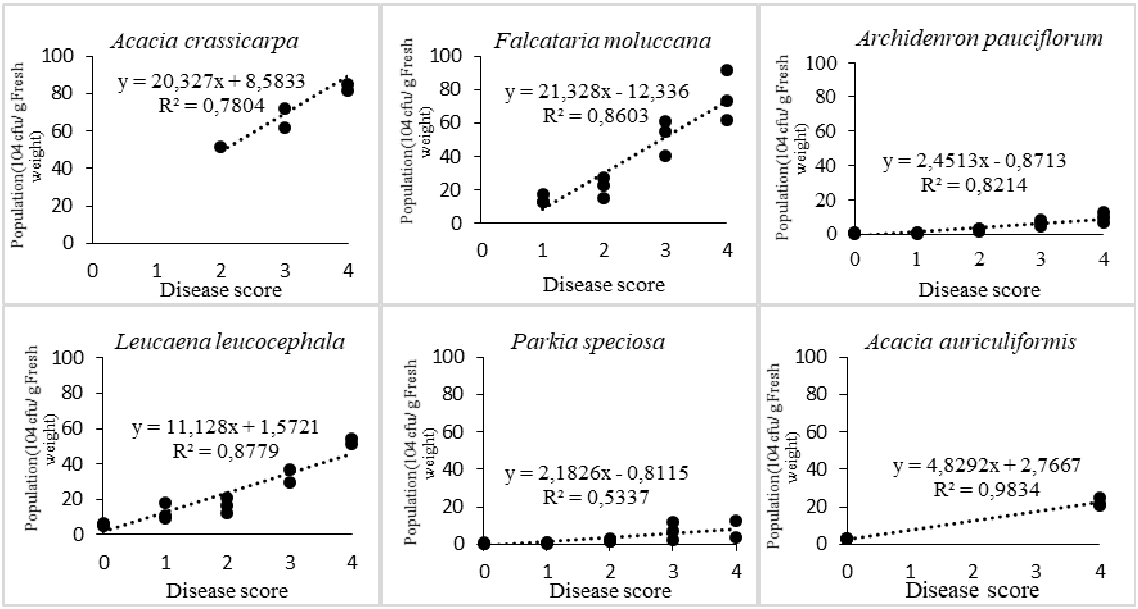


**Figure 8.** Regression analyses of disease score rate and *F. oxysporum* population.

**Table 2.** *Fusarium oxysporum* populations on root in each disease index

| Plant species | Population of *Fusarium oxysporum* (×$10^4$ CFU/g fresh weight) [a] | | | | | Average [c] |
|---|---|---|---|---|---|---|
| | 0 [b] | 1 | 2 | 3 | 4 | |
| **AF01** [d] | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | n.s | 85,13 a[e] | 85,13 |
| *Falcataria moluccana* | n.s | 17,77 a | 22,77 a | 60,98 a | 91,87 a | 76,50 |
| *Archidendron pauciflorum* | 0,45 b | 1,10 b | 3,22 b | 8,15 b | 12,53 cd | 5,06 |
| *Leucaena leucocephala* | 6,17 a | 18,10 a | 20,93 a | n.s | 51,67 b | 22,13 |
| *Parkia speciosa* | 0,32 b | 0,45 b | 2,58 b | 7,27 b | 3,57 d | 2,16 |
| *Acacia auriculiformis* | 2,92 a | n.s | n.s | n.s | 24,53 c | 4,65 |
| **BF05** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | 51,80 a | 72,08 a | 105,10 a | 92,61 |
| *Falcataria moluccana* | n.s | 13,22 a | 15,32 b | 40,33 b | 61,67 b | 43,85 |
| *Archidendron pauciflorum* | 0,47 c | 0,63 b | 1,73 c | 6,88 c | 9,90 d | 3,60 |
| *Leucaena leucocephala* | 4,67 a | 9,02 a | 12,32 b | 29,32 b | n.s | 11,16 |
| *Parkia speciosa* | 0,48 c | 0,57 b | 1,27 c | 2,33 d | n.s | 0,87 |
| *Acacia auriculiformis* | 2,55 b | n.s | n.s | n.s | 20,43 c | 3,98 |
| **DF11** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | 61,92 a | 82,00 a | 81,20 |
| *Falcataria moluccana* | n.s | 12,50 a | 27,47 a | 54,93 a | 73,00 a | 47,93 |
| *Archidendron pauciflorum* | 0,35 c | 0,35 b | 3,37 c | 4,42 c | 6,92 e | 2,19 |
| *Leucaena leucocephala* | 5,58 a | 11,17 a | 16,53 b | 36,63 b | 54,27 b | 19,69 |
| *Parkia speciosa* | 0,25 c | 0,48 b | 1,58 c | 11,97 d | 12,27 d | 5,79 |
| *Acacia auriculiformis* | 2,83 b | n.s | n.s | n.s | 21,28 c | 5,05 |

n.s: No sample, cfu: colony-forming unit

[a] *F. oxysporum* populations were calculated at the end of the experiment (21 days after inoculation).

[b] DI 0-4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, dan 4 respectively: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

**Table 3.** *Fusarium oxysporum* population average and diseases index of plant

| Plant species | Population average (×10$^4$ CFU/g fresh weight) [a] | | | Disease index [b] | | |
|---|---|---|---|---|---|---|
| | AF01 [c] | BF05 | DF11 | AF01 | BF05 | DF11 |
| *Acacia crassicarpa* | 85,13 | 92,61 | 81,20 | 4.00 | 3.48 | 3.96 |
| *Falcataria moluccana* | 76,50 | 43,85 | 47,93 | 3.44 | 3.04 | 2.80 |
| *Archidendron pauciflorum* | 5,06 | 3,60 | 2,19 | 1.96 | 1.88 | 1.40 |
| *Leucaena leucocephala* | 22,13 | 11,16 | 19,69 | 1.52 | 1.56 | 1.68 |
| *Parkia speciosa* | 2,16 | 0,87 | 5,79 | 1.80 | 1.04 | 2.16 |
| *Acacia auriculiformis* | 4,65 | 3,98 | 5,05 | 0.36 | 0.40 | 0.60 |

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, dan 4 respectively: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.

[b] DI 0-4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] *F. oxysporum* isolates.

## DISCUSSION

A recent study reported an extraordinary incidence of seedling wilt disease caused by the fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* (Soleha et al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information on the plant species that have the potential to become alternative hosts (Sampaio et al. 2021) or main hosts for the pathogen.

The results indicated that the *F. oxysporum* pathogen, which causes vascular wilt in the *A. mangium* nursery, can also infect Fabaceae plants with various host responses, as confirmed by the Koch Postulate test. *A. crassicarpa* and *F. moluccana* showed a highly susceptible reaction, while *A. pauciflorum*, *L. leucocephala,* and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen infection caused wilting symptoms in all test plant species by DI of 4.00. Although the DI was low (0.36) for *A. auriculiformis*, it still had the potential to cause plant damage if *F. oxysporum* managed to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown on contaminated soil, high exposure occurs, which leads to damage. A similar incident was reported by Pastrana et al. (2017), in which *F. oxysporum* from blackberry also caused sudden death in strawberries; therefore, it becomes a threat when planted on adjacent land. Another study also discovered that *F. oxysporum* from cactus causes root and stem rot in euphorbia when planted and cultivated on the same land (Bertetti et al. 2017). Furthermore, *P. speciosa*, which showed wilting of more than 50%, had a low severity score (as in the case of *A. auriculiformis*), which means low DI should be noted also.

The results revealed that several types of plants that were tested from the Fabaceae family had great potential to become an alternative host and even the main host for *F. oxysporum* when planted in the same field. The spread of this pathogen to a wider area allows interactions with new plants in the future (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, planting new species affected the occurrence of new outbreaks because the pathogenic strains had adapted to the soil and had become virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens' proliferation and adaptation to other plants.

The pathogen populations in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* grew slowly. The relationship between the pathogens ($g^{-1}$ fresh weight) and the DI level is as follows: i) high pathogen populations and high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population and moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population and low DI (*P. speciosa* and *A. auriculiformis*).

In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern was common where high disease scores were also found in the high population. Scott et al. (2014) reported that susceptible lettuce cultivars showed high Fusarium population levels and the vulnerable black bean genotype showed a population level of $15.4 \times 10^5$ CFU $g^{-1}$ of fresh weight (de Borba et al. 2017). In the second pattern, the population was moderate in a moderate susceptible host (*L. leucocephala*). This also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a fairly high number of pathogens on the roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* that could have been caused by a moderate pathogen infection, but the pathogen population was low. This might be due to the plant's defence mechanism. Several studies on resistant cultivars have corroborated this; Scott et al. (2014), for example, reported that resistant pepper plants also support the

pathogen's development in roots, even without external symptoms. Similar phenomena were reported by Muslim et al. (2003a) who noted that some tomato plants could be infected moderately (score 1-2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than the other plants in the same score.

The pathogen infection on *Parkia speciosa* and *A. auriculiformis* was low and the total population was also low. This indicated that the plant belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogens' colonisation and prevents the invasion of root vascular tissue. The tissue penetration by hyphae was limited to the epidermis, and the pathogens did not reach the vascular tissue. Also, the results of a study conducted by Van Den Berg et al. (2007) on banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the induction of cell wall-associated phenolic compounds. Moreover, Jiménez-Fernández et al. (2013) also reported that *Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of the root cortex and failed to reach the xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proven to be an alternative host of *F. oxysporum*. Meanwhile, *L. leucocephala*, *A. pauciflorum*, *P. speciosa*, and *A. auriculiformis* had potential as alternative hosts. Many plants of the Fabaceae family were attacked by formae specialis *F. oxysporum, such as, Vigna angularis* (*F. oxysporum* f. sp. *adzukicola*), *Cicer arietinum, Cicer* spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp. *koae*), *Lens culinaris, L. esculenta* (*F. oxysporum* f. sp. *lentis*), *Medicago sativa* (*F. oxysporum* f. sp. *medicaginis*), *Phaseolus vulgaris, P. coccineus* (*F. oxysporum* f. sp. *phaseoli*), *Pisum sativum, Cicer arietinum* (*F. oxysporum* f. sp. *pisi*), and others (Edel-Hermann and Lecomte 2019). However, in this study, *F. oxysporum* isolated from *A. mangium* has a wide host range from the Fabaceae family; therefore, it is not classified as formae specialis.

## CONCLUSION

*F. oxysporum* from *A. mangium* causes disease infection in several types of forest and industrial plants. Therefore, since it has a wide host range, it is not classified as part of the formae specialis group.

## 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, Ahmad Muslim, with the number 0124/UN9/ SB3.LP2M.PT/2020.

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# 2.Bukti konfirmasi review dan hasil review pertama (17 November 2021)

**Round 1 Status**

A review is overdue.

## Notifications

| | |
|---|---|
| [biodiv] Editor Decision | 2021-11-17 06:12 AM |
| [biodiv] Editor Decision | 2021-11-28 11:26 PM |
| [biodiv] Editor Decision | 2021-12-04 05:51 AM |
| [biodiv] Editor Decision | 2021-12-16 03:36 AM |
| [biodiv] Editor Decision | 2021-12-16 01:09 PM |

## Reviewer's Attachments

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## Review Discussions

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| Name | From | Last Reply | Replies | Closed |
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| Manuscript Revision 9450-52530-1-5-20211117 | amuslim 2021-11-26 01:25 AM | - | 0 | |
| Manuscript Revision A-9450-Article Text-53137-1-4-20211126 | amuslim 2021-12-01 02:41 PM | - | 0 | |
| Manuscript Revision A-9450-Article Text-53474-1-4-20211201 | amuslim 2021-12-05 03:01 PM | - | 0 | |
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| BILLING | dewinurpratiwi 2021-12-07 09:38 AM | dewinurpratiwi 2021-12-08 04:07 AM | 2 | |

Notifications

# [biodiv] Editor Decision

2021-11-17 06:12 AM

Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Our decision is: Revisions Required

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Reviewer A:

Comments:

1. Write references according to journal rules.

* Akond MA, Jahan MN, Sultana N, Rahman F. 2016. Effect of temperature, pH and NaCl on the isolates of actinomycetes from straw and compost samples from Savar, Dhaka, Bangladesh. Am J Microbiol and Immunol 1: 10-15.

* Barbosa J, Albano H, Silva B, Almeida MH, Nogueira T, Teixeira P. 2021. Characterization of a *Lactiplantibacillus plantarum* R23 isolated from arugula by whole-genome sequencing and its bacteriocin production ability. Int J Environ Res Public Health 18: 5515.

1. Some lines are very confusing so please correct them.
2. You haven't written any reference in the text so, please add references in text.
3. Please correct figure 8. There should be point not comma.
4. Table 1. did not show disease incidence so please check it.
5. Please read paper carefully.

Recommendation: See Comments

---

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

# [biodiv] Editor Decision

**Smujo Editors** <smujo.id@gmail.com> Wed, Nov 17, 2021 at 1:12 PM
To: Soleha Soleha <soleha057@gmail.com>, Ahmad Muslim <a_muslim@unsri.ac.id>, Suwandi Suwandi <Suwandi@fp.unsri.ac.id>, Sabaruddin Kadir <sabar@pps.unsri.ac.id>, Rahmat Pratama <rahmatpratama@pps.unsri.ac.id>

Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Our decision is: Revisions Required

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Reviewer A:

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1. Some lines are very confusing so please correct them.
2. You haven't written any reference in the text so, please add references in text.
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4. Table 1. did not show disease incidence so please check it.
5. Please read paper carefully.

Recommendation: See Comments

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Biodiversitas Journal of Biological Diversity

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# 3.Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (26 November 2021)

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

# [biodiv] Editor Decision

**a. muslim unsri** <a_muslim@unsri.ac.id> Fri, Nov 26, 2021 at 8:56 AM
To: Smujo Editors <smujo.id@gmail.com>

November 26, 2021

Dear Editor in Chief
Biodiversitas Journal of Biological Diversity

Thank you very much for your email regarding reviewer's recommendation, suggestion, and revision of our manuscript. We would like to thank and appreciate for all reviewers' suggestions and corrections.

We have made corrections and some modification according to Reviewer's revisions. Here, we enclose our revised manuscript with tracked changes and highlight of the manuscript entitled "Host Range Studies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of *Acacia mangium*" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

In this opportunity, we would like to re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Below is a summary of our changes made in response to the reviewer's comments.

1. ***Reviewer's comment:*** Write references according to journal rules.
* Akond MA, Jahan MN, Sultana N, Rahman F. 2016. Effect of temperature, pH and NaCl on the isolates of actinomycetes from straw and compost samples from Savar, Dhaka, Bangladesh. Am J Microbiol and Immunol 1: 10-15.

* Barbosa J, Albano H, Silva B, Almeida MH, Nogueira T, Teixeira P. 2021. Characterization of a Lactiplantibacillus plantarum R23 isolated from arugula by whole-genome sequencing and its bacteriocin production ability. Int J Environ Res Public Health 18: 5515.

**Our response:** Actually, we have written the refence according to journal rules. We check and recheck again, there are a few mistakes as reviewer's comments. We have revised and changed our manuscript.

2. ***Reviewer's comment:*** Some lines are very confusing so please correct them

**Our response:** We changed the sentences as follows.

***Reviewer's comment:*** Introduction, line 20: Write the authenticity of plant.
**Our response:** The authenticity of plant has been added in manuscript. (p: 1, line: 19 of revised manuscript)

***Reviewer's comment:*** Materials and method, line 44-45: Rewrite this line. Or remove this line "were utilised; they were chosen because a previous study (had described them as the most pathogenic to *A. mangium"*
**Our response:** We are appreciating for this comment, we agree and remove the sentence. (p: 2, line: 47 of revised manuscript)

***Reviewer's comment:*** Materials and method, line 64: Write the author name, rewrite it "the method designed by and modified using a disease index"
**Our response:** We have rewritten and changed the manuscript with "the method designed by Muslim et al. (2003a) and modified using a disease index "(p: 2, line: 66)

-

***Reviewer's comment:*** Materials and method, line 76: Correct the name of media "Peptone PCNB agar media".
**Our response:** The name of media is "Peptone PCNB agar Media (PPA/Nash Snyder Medium)" according to Leslie and Summerell (2006). (p: 2, line: 77-78 of revised manuscript)

***Reviewer's comment:*** Materials and method, line 79: I can't understand the meaning of this line what do you want to record "recorded based on the disease severity". Rewrite it.

**Our response:** The changed has been made to be "The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the diseases severity level. (p: 2, line: 79-81 of revised manuscript)

***Reviewer's comment:*** Results, figure7. line 113: Write the advance symptoms.
**Our response:** We have changed description of Figure 7 and modified the picture (p: 5, line 131-132 of revised manuscript)

***Reviewer's comment:*** Results, line 119: This 16% disease incidence was not mentioned in table 1.
**Our response:** Data of diseases incidence is not shown in the manuscript. We remove the sentence "and 16% incidence (p: 5, line: 138 of revised manuscript)

***Reviewer's comment :*** Result, line 123 "Although the three isolates had different tef1 genetic sequencing, they had similar virulence patterns". I removed this line because you did not mention the sequencing of isolates.
**Our response:** We agree and remove the sentence "Although the three isolates had different tef1 genetic sequencing, they had similar virulence patterns". (p: 5, line 142 of revised manuscript)

***Reviewer's comment :*** Results, line 140: I can't understand it. Rewrite it. What samples?
**Our response:** We change and rewrite the sentence from "This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. auriculiformis*" to be "This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. Auriculiformis* for DI 2 and 3". (p: 5, line: 137-138 of revised manuscript).

***Reviewer's comment :*** Results, line 140-141: I can't understand it. Rewrite it.
**Our response:** We change and rewrite the sentence to be "In DI 1, the highest population was recorded in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample for DI 1". (p: 5, line: 138-139 of revised manuscript).

***Reviewer's comment :*** Discussion, line 179-180: Which recent study and from where this is reported. Correct it.
**Our response:** The sentence has been corrected to be "A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021) (p: 8, line 180-181 of revised manuscript).

***Reviewer's comment :*** Discussion, line 190-191: Write this sentence properly.
**Our response:** We change the sentences to be "A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot also causes diseases on Euphorbia (Bertetti et al. 2017)". (p: 8, line 192-193 of revised manuscript).

***Reviewer's comment :*** Discussion, line 207-208: Please rewrite this line. Is this line can be written like this "This pattern was common where the population of the pathogen was also higher with the disease scores".
**Our response:** We agree and are grateful for this suggestion, the sentence has been changed to be "This pattern was common where the population of the pathogen was also higher with the disease scores". (p: 8, line 204-205 of revised manuscript).

***Reviewer's comment :*** Discussion, line 209-210: Rewrite this line. I can't understand the meaning "In the second pattern, the population was moderate in a moderate susceptible host (*L. leucocephala*)".
**Our response:** The sentence has been re-written to be "The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score". (p: 8, line: 207-209 of revised manuscript).

***Reviewer's comment :*** Discussion, line: 213-214: Rewrite this line. I can't understand the meaning: "A special pattern occurred on *A. pauciflorum* that could have been caused by a moderate pathogen infection, but the pathogen population was low".
**Our response:** The sentence has been re-written to be "A special pattern occurred on *A. pauciflorum* that *F. oxysporum* caused a moderate infection, but the pathogen population was low". (p: 8, line: 212-213 of revised manuscript).

***Reviewer's comment :*** Discussion, line 226: Check it again : "race 0".
**Our response:** The word (race 0) is correct (Jiménez-Fernández et al. (2013)).

**3.** ***Reviewer's comment:*** You haven't written any reference in the text so, please add references in text.
**Our response:** We do apologize for the mistake of references that we write in the Mendeley format automatically. Therefore, it can't be read by reviewer. All of the reference has been added in manuscript. (p: 1-10, line: 19-314 of revised manuscript).

4. ***Reviewer's comment:*** Please correct figure 8. There should be point not comma.

**Our response:** We have revised figure 8 by changing comma to point.

***Reviewer's comment :*** Results, line 156: Y= 20.327 and 8.5833 there should be point and comma, so please correct it all the rest of the figures 8.
**Our response:** The changed has been made on figure 8. in manuscript. (p: 6, line; 156-157 of revised manuscript)

***5.*** ***Reviewer's comment:*** Table 1. did not show disease incidence so please check it.

***Reviewer's comment:*** Results, line 118, description of Table 1 : Here you write severity and in text you wrote incidence. Check it again.
**Our response:** We are appreciating for this comment, we record diseases severity and modified the sentence for description of Table 1, from "Pathogenicity and disease severity of *Fusarium oxysporum* isolated from *Acacia mangium* "to "Disease severity and host responses to *Fusarium oxysporum* isolated from *Acacia mangium.* (p: 5, line 120 of revised manuscript).

**6.** ***Reviewer's comment:*** Please read paper carefully. Recommendation: See Comments

**Our response:** We have read our paper carefully and we changed all the words/sentences as reviewer's comment, suggestion and revision.

We feel that these changes have adequately addressed the comments and suggestions of the reviewers, and we look forward to publication in the Biodiversitas Journal of Biological Diversity.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim
Senior lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id

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## Manuscript Revision 9450-52530-1-5-20211117

### Participants Edit

Smujo Editors (editors)

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### Messages

| Note | From |
| --- | --- |
| November 26, 2021 | amuslim<br>2021-11-26<br>01:25 AM |

Dear Editor in Chief

Biodiversitas Journal of Biological Diversity

Thank you very much for your email regarding reviewer's recommendation, suggestion, and revision of our manuscript. We would like to thank and appreciate for all reviewers' suggestions and corrections.

We have made corrections and some modification according to Reviewer's revisions. Here, we enclose our revised manuscript with tracked changes and highlight of the manuscript entitled "Host Range Studies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of *Acacia mangium*" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

In this opportunity, we would like to re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Below is a summary of our changes made in response to the

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In this opportunity, we would like to re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Below is a summary of our changes made in response to the reviewer's comments.

1. ***Reviewer's comment:*** Write references according to journal rules.
* Akond MA, Jahan MN, Sultana N, Rahman F. 2016. Effect of temperature, pH and NaCl on the isolates of actinomycetes from straw and compost samples from Savar, Dhaka, Bangladesh. Am J Microbiol and Immunol 1: 10-15.

* Barbosa J, Albano H, Silva B, Almeida MH, Nogueira T, Teixeira P. 2021. Characterization of a Lactiplantibacillus plantarum R23 isolated from arugula by whole-genome sequencing and its bacteriocin production ability. Int J Environ Res Public Health 18: 5515.

**Our response:** Actually, we have written the refence according to journal rules. We check and recheck again, there are a few mistakes as reviewer's comments. We have revised and changed our manuscript.

2. ***Reviewer's comment:*** Some lines are very confusing so please correct them

**Our response:** We changed the sentences as follows.

***Reviewer's comment:*** Introduction, line 20: Write the authenticity of plant.
**Our response:** The authenticity of plant has been added in manuscript. (p: 1, line: 19 of revised manuscript)

***Reviewer's comment:*** Materials and method, line 44-45: Rewrite this line. Or remove this line "were utilised; they were chosen because a previous study (had described them as the most pathogenic to *A. mangium*"
**Our response:** We are appreciating for this comment, we agree and remove the sentence. (p: 2, line: 47 of revised manuscript)

***Reviewer's comment:*** Materials and method, line 64: Write the author name, rewrite it "the method designed by and modified using a disease index"
**Our response:** We have rewritten and changed the manuscript with "the method designed by Muslim et al. (2003a) and modified using a disease index "(p: 2, line: 66)

***Reviewer's comment:*** Materials and method, line 76: Correct the name of media "Peptone PCNB agar media".
**Our response:** The name of media is "Peptone PCNB agar Media (PPA/Nash Snyder Medium)" according to Leslie and Summerell (2006). (p: 2, line: 77-78 of revised manuscript)

***Reviewer's comment:*** Materials and method, line 79: I can't understand the meaning of this line what do you want to record "recorded based on the disease severity". Rewrite it.

**Our response:** The changed has been made to be "The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the diseases severity level. (p: 2, line: 79-81 of revised manuscript)

***Reviewer's comment:*** Results, figure7. line 113: Write the advance symptoms.
**Our response:** We have changed description of Figure 7 and modified the picture (p: 5, line 131-132 of revised manuscript)

***Reviewer's comment:*** Results, line 119: This 16% disease incidence was not mentioned in table 1.
**Our response:** Data of diseases incidence is not shown in the manuscript. We remove the sentence "and 16% incidence (p: 5, line: 138 of revised manuscript)

***Reviewer's comment :*** Result, line 123 "Although the three isolates had different tef1 genetic sequencing, they had similar virulence patterns". I removed this line because you did not mention the sequencing of isolates.
**Our response:** We agree and remove the sentence "Although the three isolates had different tef1 genetic sequencing, they had similar virulence patterns". (p: 5, line 142 of revised manuscript)

***Reviewer's comment :*** Results, line 140: I can't understand it. Rewrite it. What samples?
**Our response:** We change and rewrite the sentence from "This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. auriculiformis*" to be "This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. Auriculiformis* for DI 2 and 3". (p: 5, line: 137-138 of revised manuscript).

***Reviewer's comment :*** Results, line 140-141: I can't understand it. Rewrite it.
**Our response:** We change and rewrite the sentence to be "In DI 1, the highest population was recorded in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample for DI 1". (p: 5, line: 138-139 of revised manuscript).

***Reviewer's comment :*** Discussion, line 179-180: Which recent study and from where this is reported. Correct it.

**Our response:** The sentence has been corrected to be "A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021) (p: 8, line 180-181 of revised manuscript).

***Reviewer's comment :*** Discussion, line 190-191: Write this sentence properly.
**Our response:** We change the sentences to be "A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot also causes diseases on Euphorbia (Bertetti et al. 2017)". (p: 8, line 192-193 of revised manuscript).

***Reviewer's comment :*** Discussion, line 207-208: Please rewrite this line. Is this line can be written like this "This pattern was common where the population of the pathogen was also higher with the disease scores".
**Our response:** We agree and are grateful for this suggestion, the sentence has been changed to be "This pattern was common where the population of the pathogen was also higher with the disease scores". (p: 8, line 204-205 of revised manuscript).

***Reviewer's comment :*** Discussion, line 209-210: Rewrite this line. I can't understand the meaning "In the second pattern, the population was moderate in a moderate susceptible host (*L. leucocephala*)".
**Our response:** The sentence has been re-written to be "The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score". (p: 8, line: 207-209 of revised manuscript).

***Reviewer's comment :*** Discussion, line: 213-214: Rewrite this line. I can't understand the meaning: "A special pattern occurred on *A. pauciflorum* that could have been caused by a moderate pathogen infection, but the pathogen population was low".
**Our response:** The sentence has been re-written to be "A special pattern occurred on *A. pauciflorum* that *F. oxysporum* caused a moderate infection, but the pathogen population was low". (p: 8, line: 212-213 of revised manuscript).

***Reviewer's comment :*** Discussion, line 226: Check it again : "race 0".
**Our response:** The word (race 0) is correct (Jiménez-Fernández et al. (2013)).

**3.** ***Reviewer's comment:*** You haven't written any reference in the text so, please add references in text.
**Our response:** We do apologize for the mistake of references that we write in the Mendeley format automatically. Therefore, it can't be read by reviewer. All of the reference has been added in manuscript. (p: 1-10, line: 19-314 of revised manuscript).

4. ***Reviewer's comment:*** Please correct figure 8. There should be point not comma.

**Our response:** We have revised figure 8 by changing comma to point.

***Reviewer's comment :*** Results, line 156: Y= 20.327 and 8.5833 there should be point and comma, so please correct it all the rest of the figures 8.

**Our response:** The changed has been made on figure 8. in manuscript. (p: 6, line; 156-157 of revised manuscript)

***5. Reviewer's comment:*** Table 1. did not show disease incidence so please check it.

***Reviewer's comment:*** Results, line 118, description of Table 1 : Here you write severity and in text you wrote incidence. Check it again.
**Our response:** We are appreciating for this comment, we record diseases severity and modified the sentence for description of Table 1, from "Pathogenicity and disease severity of *Fusarium oxysporum* isolated from *Acacia mangium* "to "Disease severity and host responses to *Fusarium oxysporum* isolated from *Acacia mangium.* (p: 5, line 120 of revised manuscript).

**6. *Reviewer's comment:*** Please read paper carefully. Recommendation: See Comments

**Our response:** We have read our paper carefully and we changed all the words/sentences as reviewer's comment, suggestion and revision.

We feel that these changes have adequately addressed the comments and suggestions of the reviewers, and we look forward to publication in the Biodiversitas Journal of Biological Diversity.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim
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Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
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# Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*

**Abstract.** *Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-α) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant . The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

**Keyword:** *Acacia mangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

## INTRODUCTION

*Acacia mangium* (Willd.....) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which ,, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019)(.....). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asif et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusarium oxysporum* is an important pathogenic fungus that causes wilt disease in different plants all over the world. Soleha et al. (2021) ..... reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others. (.....).

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). ... According to Leslie and Summerell (2006)....., more than 100 formae species and races have been identified and are widespread in the world.

Besides *A. mangium,* which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana,* and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum,* causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

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## MATERIALS AND METHOD

### Fungal isolates

Three pathogenic isolates of *F. oxysporum* (AF01, BF05, and DF11), were selected, which were differentiated according to their *tef* 1-α sequence ~~(, were utilised; they were chosen because a previous study ( had described them as the most pathogenic to *A. mangium* (~~Figure 1). Isolates were cultured on~~ PDB liquid medium (potato dextrose broth) and incubated at 26-28 °C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered using two layers of sterile gauze to separate the conidia and hyphae. The conidial concentration was determined using a hemocytometer and then adjusted to a concentration of $10^6$ $ml^{-1}$ for pathogenicity test.

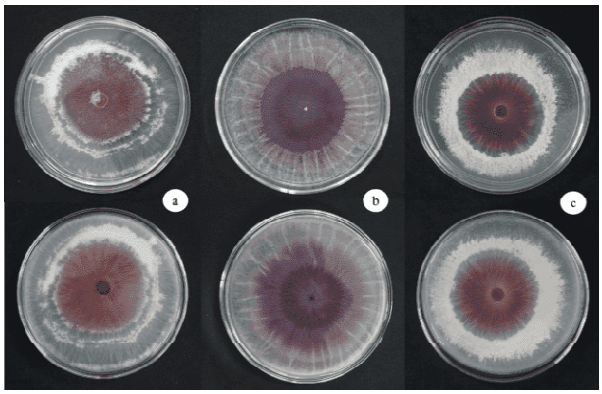


**Figure 1.** *F. oxysporum* isolates on PDA medium. (a) AF01, (b) BF05, and (c) DF11. First line: Front view; second line: reverse view.

### Plant material

The plants used were members of the fabaceae family, namely *A. crassicarpa, A. auriculiformis*, *F. moluccana*, *A. pauciflorum*, *P. speciosa*, and *L. leucocephala*, which were one month old. The seedlings were obtained from the Forest Crops Research Institute, South Sumatra. Seedlings were transferred in a mixed medium with cocopeat (1:1) using a plastic pot of 10 cm diameter and 10 cm height, and then placed in a shade house.

### Pathogenicity test

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension ($10^6$ conidia $ml^{-1}$) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity was calculated using the method designed by Muslim et al. (2003a) ~~and~~ modified using a disease index (DI) 0-4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling. Furthermore, plant responses were grouped as, R = resistant (DI=0), MR = moderately resistant/tolerance (DI = <1), MS = moderately susceptible (DI = 1.0-2.0), S = susceptible (DI = 2.1-3.0) and HS = highly susceptible (DI = 3.1-4.0). The development of disease was observed 1–21 days after inoculation.

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### *Fusarium oxysporum* population

The population of *F. oxysporum* in the roots was calculated at the end of the experiment using the method of (Muslim et al. 2003b; Li et al. 2008; Horinouchi et al. 2011) with modifications to the surface sterilization of samples. Then the plants were grouped according to severity (disease score) and washed separately under running water to remove soil residues. After that, all plants in each score were surface sterilised using 1% sodium hypochlorite for 15 minutes, then rinsed three times with distilled water. The samples and water (1:100 w/v) were homogenised using blender at 8000 rpm for 10 minutes. Then they were filtered using two layers of sterile gauze and diluted 10 to 1000 times. The suspension was spread on Peptone PCNB agar ~~n~~Media (PPA/Nash Snyder Medium) (Leslie and Summerrel, 2006) in triplicate (five Petri dishes per replication) and incubated in dark for seven days at room temperature. The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the diseases severity level. ~~recorded based on the disease severity.~~

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## RESULTS AND DISCUSSIONNN

**Pathogenicity test**

The results showed that all the six forest plants tested had– similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die . Another symptom that appeared was sudden wilting and death of plant without changing the leaf colour, while control plants did not show any symptoms (Figure 2, 3,4,5,6 &7).

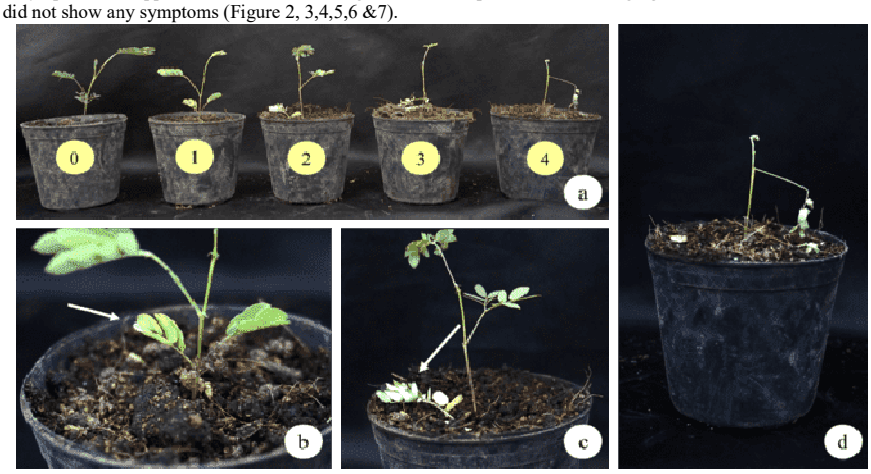


**Figure 2.** Disease index of *Acacia crassicarpa* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant.

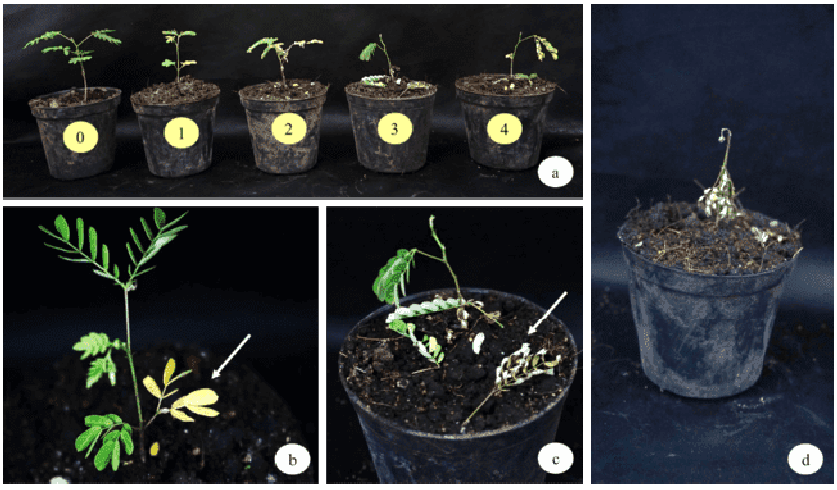


**Figure 3.** Disease index on *Falcataria moluccana* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant.

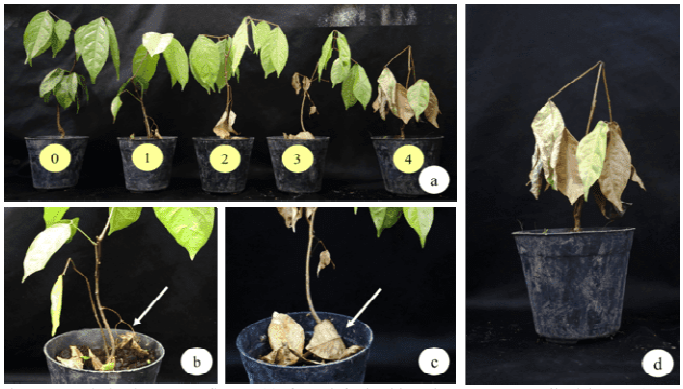


**Figure 4.** Disease index on *Archidendron pauciflorum* (a) from left: healthy plant to 100% wilted leaves (score 0−4), (b) initial symptoms: yellowing and dry from oldest leaves, (c) advanced symptoms: falling leaves, (d) dead plant.

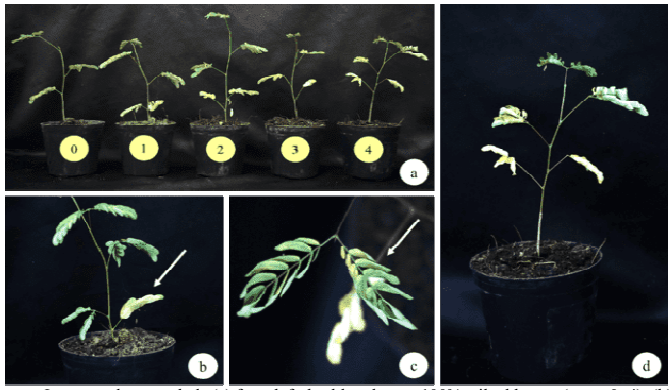


**Figure 5.** Disease index on *Leucaena leucocephala* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward.

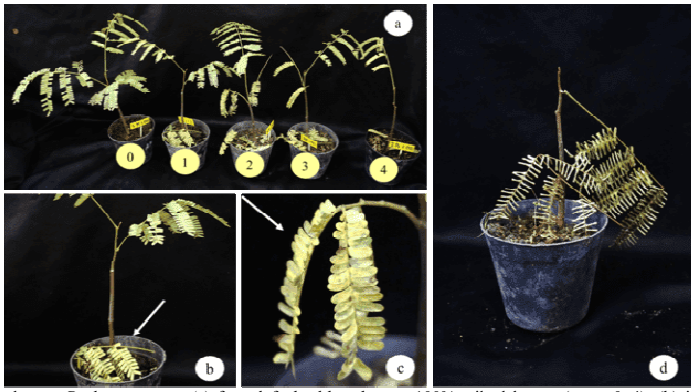


**Figure 6.** Disease index on *Parkia speciosa,* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant.

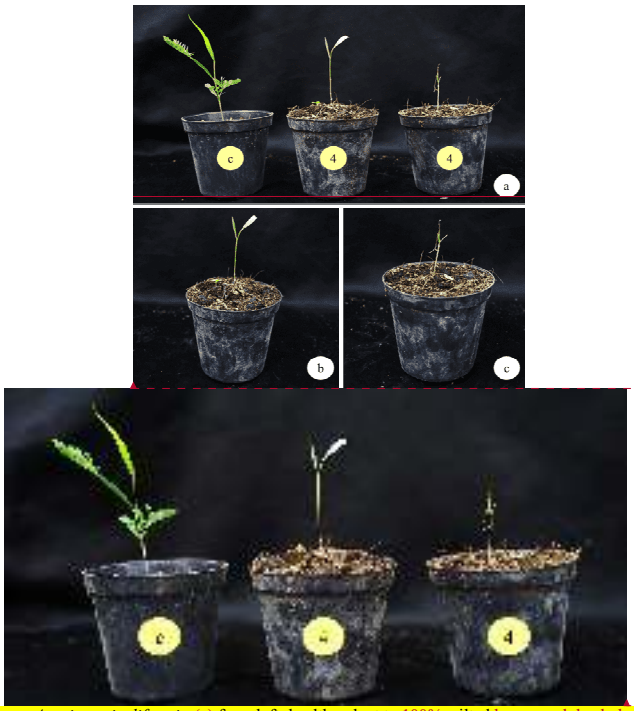


**Figure 7.** Disease index on *Acacia auriculiformis*, ~~(a)~~ from left: healthy plant to ~~100%~~ wilted ~~leaves~~ and dead plant (score 0 and ~~=~~4~~)~~; ~~(b and c) advanced symptoms: ……..~~

Disease severity was significantly higher than controls. *A. crassicarpa* and *F. moluccana* were most severely affected with an average score of 4.00 and 3.44, respectively ~~and the incidence of wilting was 100%~~. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were showed moderate disease severity i.e. 1.96, 1.68, and 1.80, respectively, whereas *A. auriculiformis* had the lowest (0.36) disease severity (Table 1) ~~and (16%) incidence ) (Table 1~~). Based on the disease score, host plants were classified into three groups: i) highly susceptible (*A. crassicarpa* and *F. moluccana*), ii) moderately susceptible (*A. pauciflorum P. speciosa*, and *L. leucocephala*), and iii) moderate resistance/tolerance (*A. auriculiformis*) (Table 1).

~~.~~ Result exhibited that there was no significant difference between the disease severity in the same host that had been inoculated with different isolates.

**Table 1.** ~~Pathogenicity and~~ disease severity and host responses to~~of~~ *Fusarium oxysporum* isolated from *Acacia mangium*

| Plant species | Isolates [a)] | | | | | |
|---|---|---|---|---|---|---|
| | AF01 [b)] | Response [c)] | BF05 | Response | DF11 | Response |
| *Acacia crassicarpa* | 4.00 a | HS [c)] | 3.48 a | HS | 3.96 a | HS |
| *Falcataria moluccana* | 3.44 ab | HS | 3.04 a | HS | 2.80 ab | S |
| *Archidendron pauciflorum* | 1.96 bc | MS | 1.88 b | MS | 1.40 cd | MS |
| *Leucaena leucocephala* | 1.52 c | MS | 1.56 b | MS | 1.68 bc | MS |
| *Parkia speciosa* | 1.80 c | MS | 1.04 bc | MS | 2.16 bc | S |
| *Acacia auriculiformis* | 0.36 d | MR | 0.40 c | MR | 0.60 d | MR |

Values followed by the same letter in each row are not significant.

[a] DI 0-4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.

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[b] *F. oxysporum* isolates.

[c] Host response were grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI = 1.0-2.0); S = susceptible (DI = 2.1-3.0); HS = highly susceptible (DI = 3.1-4.0) (Bertetti et al. 2018)

**_Fusarium oxysporum_ population**

The total population of *F. oxysporum* on the roots was determined by calculating the CFU for each category of damage. The results showed that at a score of 4, *A. crassicarpa* and *F. moluccana* showed a significantly higher population (82.00–105.10 × $10^4$ CFU $g^{-1}$ fresh weight) than other plants. The lowest population was recorded in *P. speciosa* and *A. pauciflorum* (3.57–12.27 × $10^4$ CFU $g^{-1}$ fresh weight). This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. Auriculiformis* for DI 2 and 3. In DI 1, the highest population was recorded in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample for DI 1. In ~~control plants (~~DI =0~~)~~, the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana* for DI 0(Table 2 &Table 3).

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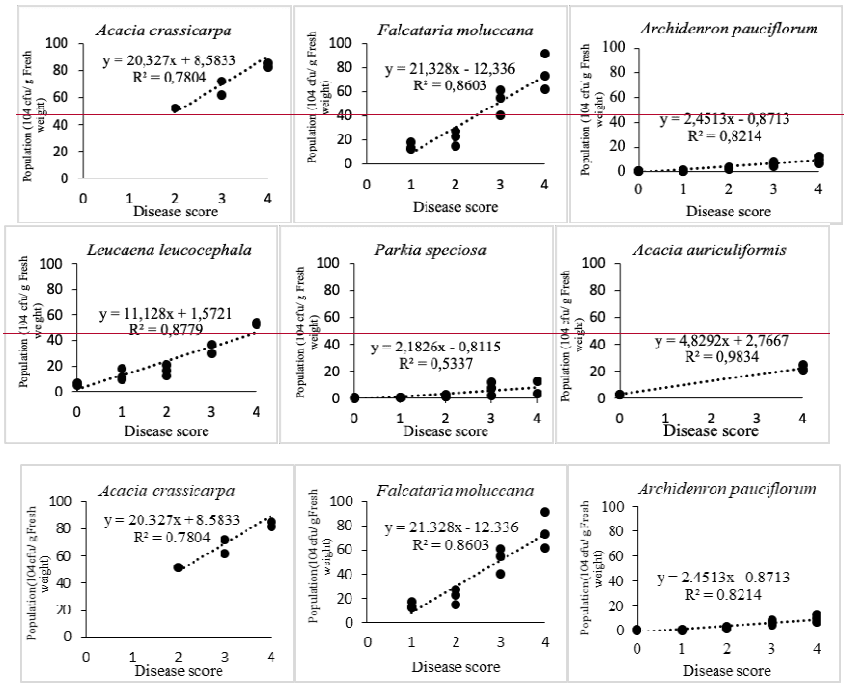
The regression analysis results showed that all plants except *P. speciosa* had a linear relationship pattern between the increase in disease score and population. The pathogenic population on *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, as indicated by the magnitude of regression gradient coefficient (m=20.3–21.3). However, moderate increase was observed in *L. leucocephala* (m=11.2) (m=11.2) and very slow in *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Table 3 showed that isolates were different in *tef1-α*, but the population and DI patterns were similar for each test plant. The correlation between the population of pathogen ($g^{-1}$ fresh weight) and the level of DI were described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).



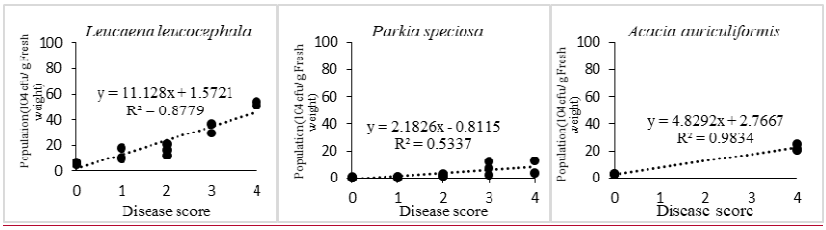



**Figure 8.** Regression analysis of disease score rate and *F. oxysporum* population

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**Table 2.** *Fusarium oxysporum* population on root in each disease index

| Plant species | Population of *Fusarium oxysporum* (×10$^4$ CFU/g fresh weight) [a] | | | | | Average [c] |
|---|---|---|---|---|---|---|
| | 0 [b] | 1 | 2 | 3 | 4 | |
| **AF01** [d] | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | n.s | 85.13 a[e] | 85.13 |
| *Falcataria moluccana* | n.s | 17.,77 a | 22.77 a | 60.98 a | 91.87 a | 76.50 |
| *Archidendron pauciflorum* | 0.45 b | 1.10 b | 3.22 b | 8.15 b | 12.53 cd | 5.06 |
| *Leucaena leucocephala* | 6.17 a | 18.10 a | 20.93 a | n.s | 51.67 b | 22.13 |
| *Parkia speciosa* | 0.32 b | 0.45 b | 2.58 b | 7.27 b | 3.57 d | 2.16 |
| *Acacia auriculiformis* | 2.92 a | n.s | n.s | n.s | 24.53 c | 4.65 |
| **BF05** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | 51.80 a | 72.08 a | 105.10 a | 92.61 |
| *Falcataria moluccana* | n.s | 13.22 a | 15.32 b | 40.33 b | 61.67 b | 43.85 |
| *Archidendron pauciflorum* | 0.47 c | 0.63 b | 1.73 c | 6.88 c | 9.90 d | 3.60 |
| *Leucaena leucocephala* | 4.67 a | 9.02 a | 12.32 b | 29.32 b | n.s | 11.16 |
| *Parkia speciosa* | 0.48 c | 0.57 b | 1.27 c | 2.33 d | n.s | 0.87 |
| *Acacia auriculiformis* | 2.55 b | n.s | n.s | n.s | 20.43 c | 3.98 |
| **DF11** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | 61.92 a | 82.00 a | 81.20 |
| *Falcataria moluccana* | n.s | 12.50 a | 27.47 a | 54.93 a | 73.00 a | 47.93 |
| *Archidendron pauciflorum* | 0.35 c | 0.35 b | 3.37 c | 4.42 c | 6.92 e | 2.19 |
| *Leucaena leucocephala* | 5.58 a | 11.17 a | 16.53 b | 36.63 b | 54.27 b | 19.69 |
| *Parkia speciosa* | 0.25 c | 0.48 b | 1.58 c | 11.97 d | 12.27 d | 5.79 |
| *Acacia auriculiformis* | 2.83 b | n.s | n.s | n.s | 21.28 c | 5.05 |

n.s: No sample, cfu: colonyforming unit

[a] *F. oxysporum* populations calculated at the end of the experiment (21 days after inoculation).

[b] DI 0-4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

**Table 3.** *Fusarium oxysporum* population average and diseases index of plant

| Plant species | Population average (×10⁴ CFU/g fresh weight) [a] | | | Disease index [b] | | |
|---|---|---|---|---|---|---|
| | AF01 [c] | BF05 | DF11 | AF01 | BF05 | DF11 |
| *Acacia crassicarpa* | 85.13 | 92.61 | 81.20 | 4.00 | 3.48 | 3.96 |
| *Falcataria moluccana* | 76.50 | 43.85 | 47.93 | 3.44 | 3.04 | 2.80 |
| *Archidendron pauciflorum* | 5.06 | 3.60 | 2.19 | 1.96 | 1.88 | 1.40 |
| *Leucaena leucocephala* | 22.13 | 11.16 | 19.69 | 1.52 | 1.56 | 1.68 |
| *Parkia speciosa* | 2.16 | 0.87 | 5.79 | 1.80 | 1.04 | 2.16 |
| *Acacia auriculiformis* | 4.65 | 3.98 | 5.05 | 0.36 | 0.40 | 0.60 |

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4 : A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.

[b] DI 0-4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] *F. oxysporum* isolates.

## Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses.. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala,* and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot also causes diseases on Euphorbia (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern was common where the population of the pathogen was also higher with the disease scores Scott et al. (2014) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotype showed a population level of 15.4 × $10^5$ CFU $g^{-1}$ (de Borba et al. 2017). The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate disease score. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a significantly moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* that *F. oxysporum* caused by a moderate pathogen infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslim et al. (2003a) who noted that some tomato plants are infected moderately (score 1-2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that planst belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the

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hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the ~~epidermis~~epidermis, then the pathogens do not reach the vascular tissue. Van Den Berg et al. (2007)~~.....~~ reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the induction of cell wall-associated phenolic compounds. Jiménez-Fernández et al. (2013) ~~.....~~ also reported that *Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proven to be an alternative host of *F. oxysporum*. Whereas *L. leucocephala*, *A. pauciflorum*, *P. speciosa*, and *A. auriculiformis* had potential as alternative hosts. Many plants of fabaceae family was attacked by formae specialis *F. oxysporum,* such as *Vigna angularis* (*F. oxysporum* f. sp. *adzukicola*), *Cicer arietinum, Cicer* spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp. *koae*), *Lens culinaris, L. esculenta* (*F. oxysporum* f. sp. *lentis*), *Medicago sativa* (*F. oxysporum* f. sp. *medicaginis*), *Phaseolus vulgaris, P. coccineus* (*F. oxysporum* f. sp. *phaseoli*), *Pisum sativum, Cicer arietinum* (*F. oxysporum* f. sp. *pisi*) (Edel-Hermann and Lecomte 2019)~~...~~. However, in this study, *F. oxysporum* isolated from *A. mangium* has a wide host range from fabaceae family; therefore, it is not classified as formae specialis.

In conclusion, *F. oxysporum* isolated from *A. mangium* causes infection in several types of forest and industrial plants. Since it has a wide host range, it is not classified as part of the formae specialis group.

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# 4.Bukti konfirmasi review dan hasil review kedua (29 November 2021)

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

# [biodiv] Editor Decision

**Smujo Editors** <smujo.id@gmail.com> Mon, Nov 29, 2021 at 6:25 AM
To: Soleha Soleha <soleha057@gmail.com>, Ahmad Muslim <a_muslim@unsri.ac.id>, Suwandi Suwandi <Suwandi@fp.unsri.ac.id>, Sabaruddin Kadir <sabar@pps.unsri.ac.id>, Rahmat Pratama <rahmatpratama@pps.unsri.ac.id>

Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Our decision is: Revisions Required

---

Reviewer A:

Comments:

1. There are still some corrections in paper, please make correction as per sugesstion.

2. Please clear the meaning of no samples.

3. read paper very carefully.

Recommendation: See Comments

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Biodiversitas Journal of Biological Diversity

**A-9450-Article Text-53137-1-4-20211126.doc**
1702K

**Notifications**

# [biodiv] Editor Decision

2021-11-28 11:26 PM

Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Our decision is: Revisions Required

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Reviewer A:

Comments:

1. There are still some corrections in paper, please make correction as per sugesstion.

2. Please clear the meaning of no samples.

3. read paper very carefully.

Recommendation: See Comments

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Biodiversitas Journal of Biological Diversity

# 5.Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit (02 Desember 2021)

## Manuscript Revision A-9450-Article Text-53137-1-4-20211126

**Participants** Edit

Smujo Editors (editors)

Ahmad Muslim (amuslim)

DEWI NUR PRATIWI (dewinurpratiwi)

Agustina Putri (aputri1)

**Messages**

Desember 1, 2021

amuslim
2021-12-01
02:41 PM

Dear Editor in Chief

Biodiversitas Journal of Biological Diversity

Thank you very much for your email regarding reviewer's recommendation, suggestion, and revision of our manuscript. We would like to thank and appreciate for all reviewers' suggestions and corrections.

We have made corrections and some modification according to Reviewer's revisions. Here, we enclose our revised manuscript with tracked changes of the manuscript entitled "Host Range Studies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of *Acacia mangium*" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

In this opportunity, we would like to re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Below is a summary of our changes made in response to the reviewer's comments.

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

# [biodiv] Editor Decision

**a. muslim unsri** <a_muslim@unsri.ac.id> Thu, Dec 2, 2021 at 10:05 AM
To: Smujo Editors <smujo.id@gmail.com>

Dear Editor in Chief
Biodiversitas Journal of Biological Diversity

Thank you very much for your email regarding reviewer's recommendation, suggestion, and revision of our manuscript. We would like to thank and appreciate for all reviewers' suggestions and corrections.

We have made corrections and some modification according to Reviewer's revisions. Here, we enclose our revised manuscript with tracked changes of the manuscript entitled "Host Range Studies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of *Acacia mangium*" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

In this opportunity, we would like to re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Below is a summary of our changes made in response to the reviewer's comments.

1. ***Reviewer's comment:*** There are still some corrections in paper, please make correction as per sugesstion.
**Our response:** We have changed the word and the sentences, that some minor corrections are changed directly in the manuscript and some corrections are as follows.

***Reviewer's comment:*** If you have followed any reference for disease index please mention the author name.
**Our response:** we have insert reference (Bertetti et al. 2018) for disease index in this line.

***Reviewer's comment:*** S stands for susceptible but in the text you have classified *Falcataria moluccana* as highly susceptible host. Please check it again.
**Our response:** We classified *Falcataria moluccana* as highly susceptible host because this plant showed highly susceptible (HS) responses against two isolates (AF01 and BF05) and only one isolate (DF11) show susceptible response (S).

***Reviewer's comment:*** S stands for susceptible but in the text you have classified *Parkia speciosa* moderate susceptible host. Please check it again.
**Our response:** We classified *Parkia speciosa* as moderate susceptible host because this plant showed moderate susceptible (MS) responses against two isolates (AF01 and BF05) and only one isolate (DF11) shows susceptible response (S).

***Reviewer's comment:*** Remove the old figures
**Our response:** We have removed the old figures

2. ***Reviewer's comment:*** Please clear the meaning of no samples.
**Our response:**
***Reviewer's comment:*** I have asked you earlier that what do you mean by no samples. Does it mean that *Fusaium* was not found in *A. auriculiformis*. Please clear it.
**Our response:** No sample means that there was not found on inoculated *Acacia auriculiformis* that showed disease index 2 and 3. All inoculated plants showed only disease index 0 and disease index 4. In this experiment we observed population of *Fusarium oxysporum* based on disease index of the inoculated plant.

***Reviewer's comment:*** Again the same question that I have asked you earlier that what do you mean by no samples. Does it mean that *Fusaium* was not found in *A. Auriculiformis* and *A. crassicarpa*. Please clear it.
**Our response:** No sample means that there was not found on inoculated *A. auriculiformis* and *A. crassicarpa* that showed disease index 1. All inoculated *A. auriculiformis* showed only diseases index 0 and 4. While *A. crassicarpa* showed only disease index 4 (AF01); disease index 2, 3, and 4 (BF05); and disease index 3 and 4 (DF11). In this experiment we observed population of *Fusarium oxysporum* based on disease index of the inoculated plant.

***Reviewer's comment:*** Confusing line "In control plants (DI=0), the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana* (Table 2 & Table 3)."
**Our response:** *Fusarium oxysporum* was not found on control uninoculated plants.
DI 0 means that disease index for inoculated *L. leucocephala, Leucaena leucocephala*, *Parkia speciosa* and *A. auriculiformis.* While, *A. crassicarpa* and *F. moluccana* did not produce DI 0 (It is mean that there was no sample for *A. crassicarpa* and *F. moluccana*), because all plants showed symptoms (Table 2). We have change "control plants" to be "inoculated plants"

3. ***Reviewer's comment:*** read paper very carefully.
**Our response:** We have read our paper carefully and we changed all the words/sentences as reviewer's comment, suggestion and revision.

We feel that these changes have adequately addressed the comments and suggestions of the reviewers, and we look forward to publication in the Biodiversitas Journal of Biological Diversity.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim
Senior lecturer
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id

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**Manuscript_Soleha_Rev2.doc**
1551K

# 6.Bukti konfirmasi review dan hasil review ketiga (04 Desember 2021)

Notifications

# [biodiv] Editor Decision

2021-12-04 05:51 AM

Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Our decision is: Revisions Required

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Reviewer A:
Recommendation: See Comments

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Biodiversitas Journal of Biological Diversity

Reviewer's Attachments Search

No Files

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

# [biodiv] Editor Decision

**Smujo Editors** <smujo.id@gmail.com> Sat, Dec 4, 2021 at 12:51 PM
To: Soleha Soleha <soleha057@gmail.com>, Ahmad Muslim <a_muslim@unsri.ac.id>, Suwandi Suwandi <Suwandi@fp.unsri.ac.id>, Sabaruddin Kadir <sabar@pps.unsri.ac.id>, Rahmat Pratama <rahmatpratama@pps.unsri.ac.id>

Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Our decision is: Revisions Required

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Reviewer A:
Recommendation: See Comments

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Biodiversitas Journal of Biological Diversity

**A-9450-Article Text-53474-1-4-20211201.doc**
1548K

# 7. Bukti konfirmasi submit revisi ketiga, respon kepada reviewer, dan artikel yang diresubmit (06 Desember 2021)

## Manuscript Revision A-9450-Article Text-53474-1-4-20211201

### Participants Edit

Smujo Editors (editors)

Ahmad Muslim (amuslim)

DEWI NUR PRATIWI (dewinurpratiwi)

Agustina Putri (aputri1)

### Messages

| Note | From |
| --- | --- |
| Desember 5, 2021 | amuslim<br>2021-12-05<br>03:01 PM |

Dear Editor in Chief

Biodiversitas Journal of Biological Diversity

Thank you very much for your email regarding reviewer's revision of our manuscript.

We have revised and edited the text and the title of figure 8 of our manuscript (Line: 146 and line 150 (figure 8 title)) as Reviewer's revision. Enclose our final revised manuscript entitled "Host Range Studies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of *Acacia mangium*" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

We hope our revision is appropriate with the reviewer's suggestion.

Here we re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

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

# [biodiv] Editor Decision

**a. muslim unsri** <a_muslim@unsri.ac.id> Mon, Dec 6, 2021 at 6:54 AM
To: Smujo Editors <smujo.id@gmail.com>

Dear Editor in Chief
Biodiversitas Journal of Biological Diversity

Thank you very much for your email regarding reviewer's revision of our manuscript.

We have revised and edited the text and the title of figure 8 of our manuscript (Line: 146 and line 150 (figure 8 title)) as Reviewer's revision. Enclose our final revised manuscript entitled "Host Range Studies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of *Acacia mangium*" by Soleha Soleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

We hope our revision is appropriate with the reviewer's suggestion.

Here we re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Please feel free to contact me if you need any additional information or clarification.
Thank you very much for your excellent cooperation

Sincerely,
Ahmad Muslim, Ph.D
Associate Professor
Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang, Indonesia
E-mail: a_muslim@unsri.ac.id
[Quoted text hidden]

**Manuscript_Soleha_Rev3.doc**
1541K

# 8. Bukti konfirmasi accepted (16 Desember 2021)

Notifications

# [biodiv] Editor Decision

2021-12-16 03:36 AM

SOLEHA SOLEHA, AHMAD MUSLIM, SUWANDI SUWANDI, SABARUDDIN KADIR, RAHMAT PRATAMA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host range studies of Fusarium oxysporum, causal agent of seedling wilt disease of Acacia mangium".

Our decision is to: Accept Submission

Best Regards,
Team Support Smujo.id

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Biodiversitas Journal of Biological Diversity

[biodiv] Editor Decision 2021-12-16 03:36 AM

[biodiv] Editor Decision 2021-12-16 01:09 PM

Reviewer's Attachments Search

No Files

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

# [biodiv] Editor Decision

**Team Support Smujo** <smujo.id@gmail.com> Thu, Dec 16, 2021 at 10:36 AM
To: SOLEHA SOLEHA <soleha057@gmail.com>, AHMAD MUSLIM <a_muslim@unsri.ac.id>, SUWANDI SUWANDI <Suwandi@fp.unsri.ac.id>, SABARUDDIN KADIR <sabar@pps.unsri.ac.id>, RAHMAT PRATAMA <rahmatpratama@pps.unsri.ac.id>

SOLEHA SOLEHA, AHMAD MUSLIM, SUWANDI SUWANDI, SABARUDDIN KADIR, RAHMAT PRATAMA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host range studies of Fusarium oxysporum, causal agent of seedling wilt disease of Acacia mangium".

Our decision is to: Accept Submission

Best Regards,
Team Support Smujo.id

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Biodiversitas Journal of Biological Diversity

# 9. Bukti konfirmasi Uncorrected proof (07 Desember 2021)

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

# [biodiv] New notification from Biodiversitas Journal of Biological Diversity

**DEWI NUR PRATIWI** <smujo.id@gmail.com> Tue, Dec 7, 2021 at 4:18 PM
Reply-To: Ahmad Dwi Setyawan <editors@smujo.id>
To: Ahmad Muslim <a_muslim@unsri.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Uncorrected Proof" regarding the submission "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Link: https://smujo.id/biodiv/authorDashboard/submission/9450

Ahmad Dwi Setyawan

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Biodiversitas Journal of Biological Diversity

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# Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*

**SOLEHA SOLEHA[1], AHMAD MUSLIM[2,♥], SUWANDI SUWANDI[2], SABARUDDIN KADIR[3], RAHMAT PRATAMA[1]**

[1] Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl. Indralaya Indah, Indralaya, Ogan Ilir Regency, Sumatra Selatan, Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Jl. Indralaya Indah, Indralaya, Ogan Ilir Regency, Sumatra Selatan, Indonesia, Tel./fax. +62-896-3874-9695 ♥email: a_muslim@unsri.ac.id

[3]Department of Soil Sciences, Faculty of Agriculture, Universitas Sriwijaya, Jl. Indralaya Indah, Indralaya, Ogan Ilir Regency, Sumatra Selatan, Indonesia

Manuscript received: xxx. Revision accepted: xxx December 2021.

**Abstract.** *Soleha S, Muslim A, Suwandi S, Kadir S, Pratama R. 2021. Host range studies of* Fusarium oxysporum*, causal agent of seedling wilt disease of* Acacia mangium*. Biodiversitas 23: xxxx. Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-□) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

**Keyword:** *Acacia mangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

## INTRODUCTION

*Acacia mangium* (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asif et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusarium oxysporum* is an important pathogenic fungus that causes wilt disease in different plants all over the world. Soleha et al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A. mangium,* which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana,* and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum,* causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

## MATERIALS AND METHOD

### Fungal isolates

Three pathogenic isolates of *F. oxysporum* (AF01, BF05, and DF11) were selected, which were differentiated according to their *tef* 1-α sequence (Figure 1). Isolates were cultured on PDB liquid medium (potato dextrose broth) and incubated at 26-28 °C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered using two layers of sterile gauze to separate the conidia and hyphae. The conidial concentration was determined using a hemocytometer and then adjusted to a concentration of $10^6$ $ml^{-1}$ for pathogenicity test.

### Plant material

The plants used were members of the fabaceae family, namely *A. crassicarpa, A. auriculiformis*, *F. moluccana*, *A. pauciflorum*, *P. speciosa*, and *L. leucocephala*, which were one month old. The seedlings were obtained from the Forest Crops Research Institute, South Sumatra. Seedlings were transferred in a mixed medium with cocopeat (1:1) using a plastic pot of 10 cm diameter and 10 cm height, and then placed in a shade house.

### Pathogenicity test

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension ($10^6$ conidia $ml^{-1}$) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity was calculated using the method of Muslim et al. (2003a) and the disease index (DI) was classified into following grades, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling (Bertetti et al. 2018). Furthermore, plant responses were grouped as, R = resistant (DI=0), MR = moderately resistant/tolerance (DI = <1), MS = moderately susceptible (DI = 1.0–2.0), S = susceptible (DI = 2.1–3.0) and HS = highly susceptible (DI = 3.1–4.0). The development of disease was observed 1–21 days after inoculation.

### *Fusarium oxysporum* population

The population of *F. oxysporum* in the roots was calculated at the end of the experiment using the method of (Muslim et al. 2003b; Li et al. 2008; Horinouchi et al. 2011) with modifications to the surface sterilization of samples. Then the plants were grouped according to severity (disease score) and washed separately under running water to remove soil residues. After that, all plants in each score were surface sterilised using 1% sodium hypochlorite for 15 minutes, then rinsed three times with distilled water. The samples and water (1:100 w/v) were homogenised using blender at 8000 rpm for 10 minutes. Then they were filtered using two layers of sterile gauze and diluted 10 to 1000 times. The suspension was spread on Peptone PCNB agar Media (PPA/Nash Snyder Medium) (Leslie and Summerell 2006) in triplicate (five Petri dishes per replication) and incubated in dark for seven days at room temperature. The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the level of diseases severity.

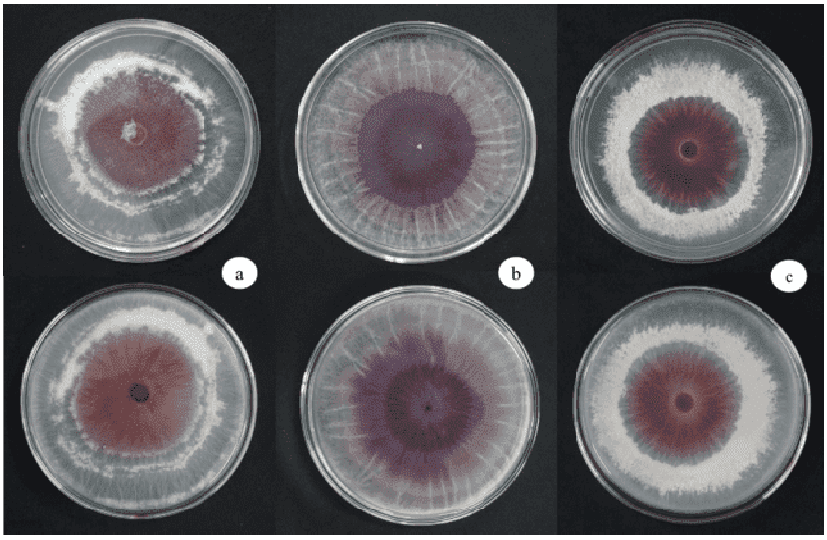


**Figure 1.** *F. oxysporum* isolates on PDA medium. (a) AF01, (b) BF05, and (c) DF11. First line: front view; second line: reverse view.

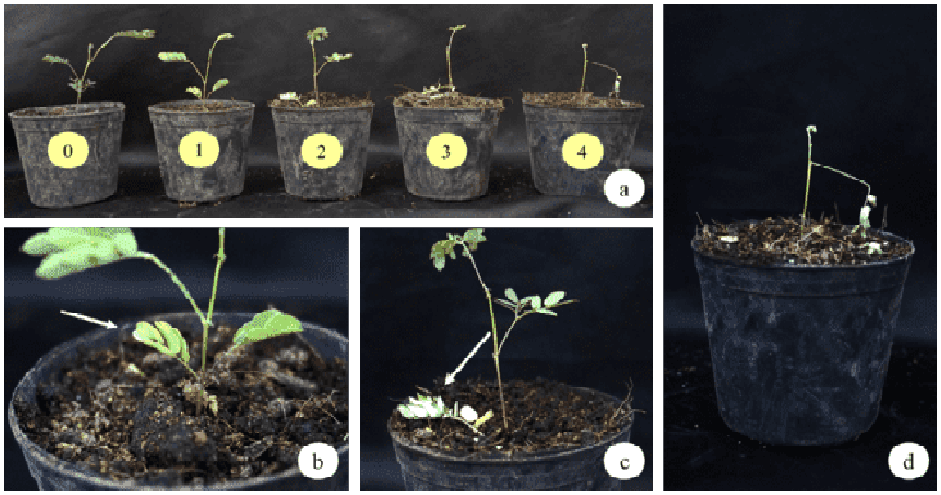


**Figure 2.** Disease index of *Acacia crassicarpa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

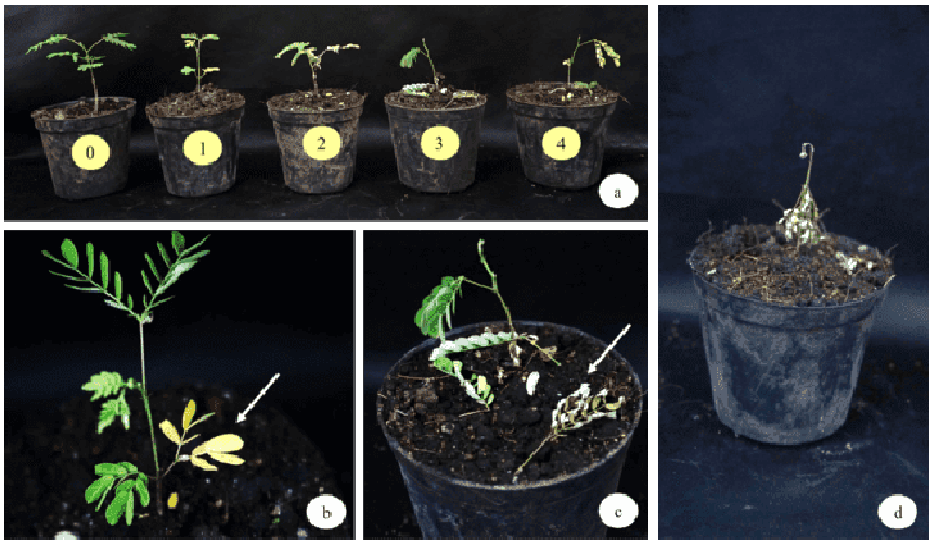


**Figure 3.** Disease index on *Falcataria moluccana*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

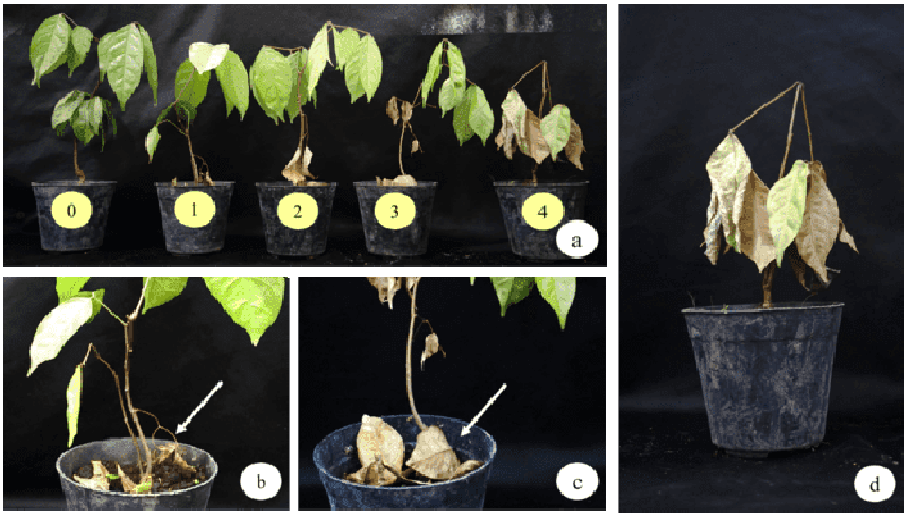


**Figure 4.** Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

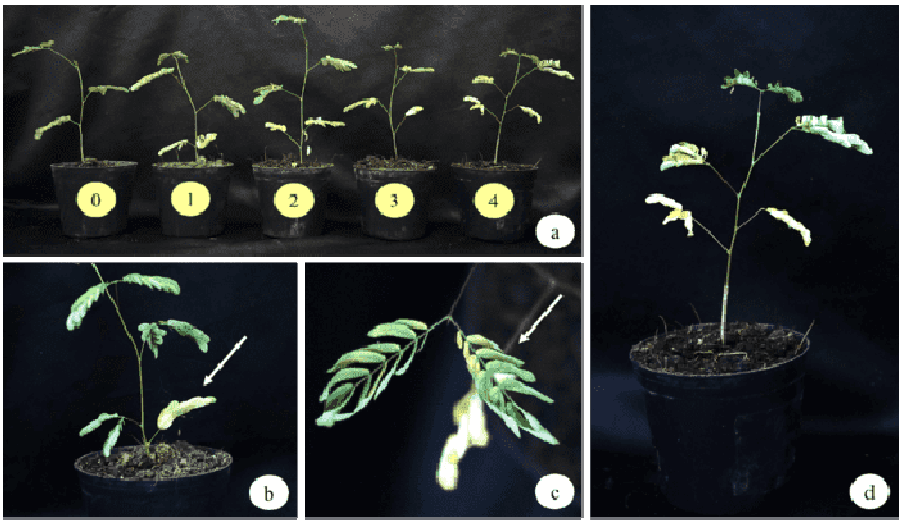


**Figure 5.** Disease index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

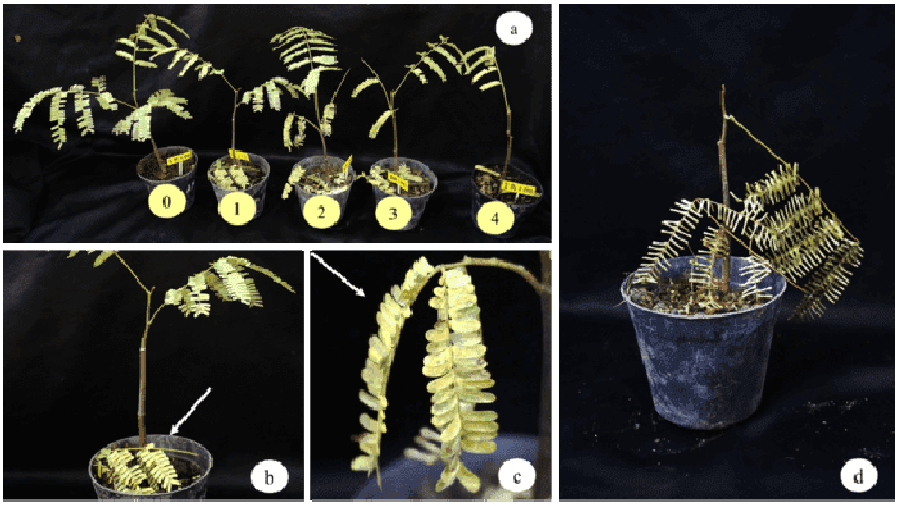


**Figure 6.** Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant


**Figure 7.** Disease index on *Acacia auriculiformis*, from left: healthy plant to wilted and dead plant (score 0–4)

## RESULTS AND DISCUSSION

### Pathogenicity test

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing the leaf colour, while control plants did not show any symptoms (Figures 2-7).

Disease severity was significantly higher than controls. *A. crassicarpa* and *F. moluccana* were most severely affected with an average score of 4.00 and 3.44, respectively. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were showed moderate disease severity i.e. 1.96, 1.68, and 1.80, respectively, whereas *A. auriculiformis* had the lowest (0.36) disease severity (Table 1). Based on the disease score, host plants were classified into three groups: i) highly susceptible (*A. crassicarpa* and *F. moluccana*), ii) moderately susceptible (*A. pauciflorum P. speciosa*, and *L. leucocephala*), and iii) moderate resistance/tolerance (*A. auriculiformis*). Result exhibited that there was no significant difference between the disease severity in the same host that had been inoculated with different isolates (Table 1).

**Table 1.** Disease severity and host responses to *Fusarium oxysporum* isolated from *Acacia mangium*

| Plant species | Isolates [a)] | | | | | |
|---|---|---|---|---|---|---|
| | AF01 [b)] | Response [c)] | BF05 | Response | DF11 | Response |
| *Acacia crassicarpa* | 4.00 a | HS [c)] | 3.48 a | HS | 3.96 a | HS |
| *Falcataria moluccana* | 3.44 ab | HS | 3.04 a | HS | 2.80 ab | S |
| *Archidendron pauciflorum* | 1.96 bc | MS | 1.88 b | MS | 1.40 cd | MS |
| *Leucaena leucocephala* | 1.52 c | MS | 1.56 b | MS | 1.68 bc | MS |
| *Parkia speciosa* | 1.80 c | MS | 1.04 bc | MS | 2.16 bc | S |
| *Acacia auriculiformis* | 0.36 d | MR | 0.40 c | MR | 0.60 d | MR |

Values followed by the same letter in each row are not significant.

[a] DI 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.

[b)] *F. oxysporum* isolates.

[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI = 1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

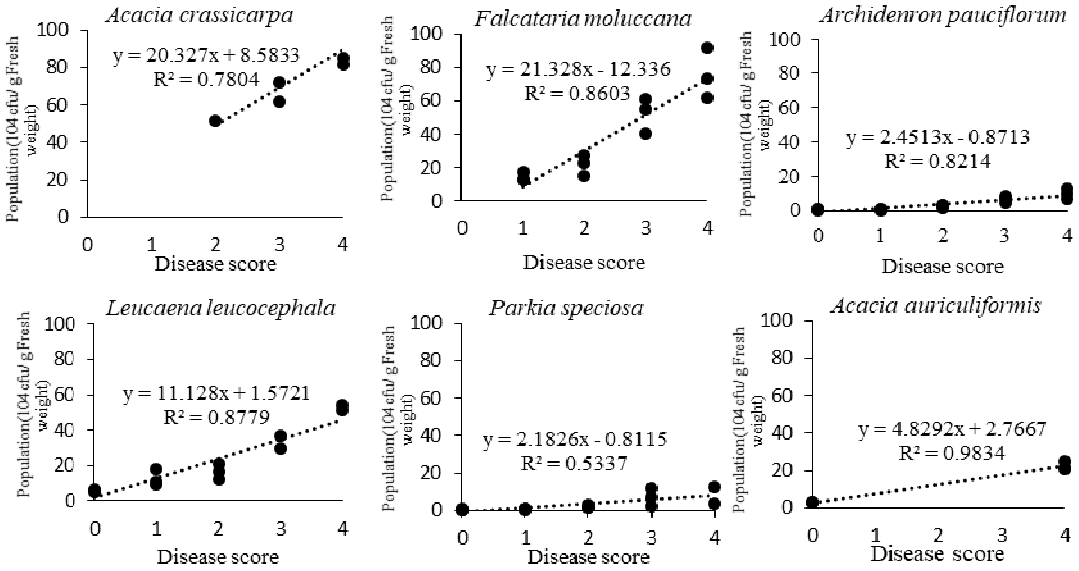


**Figure 8.** Regression analysis of disease score rate and *F. oxysporum* population

**Table 2.** *Fusarium oxysporum* population on root in each disease index

| Plant species | Population of *Fusarium oxysporum* (×10$^4$ CFU/g fresh weight) [a] | | | | | Average [c] |
|---|---|---|---|---|---|---|
| | 0 [b] | 1 | 2 | 3 | 4 | |
| **AF01** [d] | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | n.s | 85.13 a[e] | 85.13 |
| *Falcataria moluccana* | n.s | 17.77 a | 22.77 a | 60.98 a | 91.87 a | 76.50 |
| *Archidendron pauciflorum* | 0.45 b | 1.10 b | 3.22 b | 8.15 b | 12.53 cd | 5.06 |
| *Leucaena leucocephala* | 6.17 a | 18.10 a | 20.93 a | n.s | 51.67 b | 22.13 |
| *Parkia speciosa* | 0.32 b | 0.45 b | 2.58 b | 7.27 b | 3.57 d | 2.16 |
| *Acacia auriculiformis* | 2.92 a | n.s | n.s | n.s | 24.53 c | 4.65 |
| **BF05** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | 51.80 a | 72.08 a | 105.10 a | 92.61 |
| *Falcataria moluccana* | n.s | 13.22 a | 15.32 b | 40.33 b | 61.67 b | 43.85 |
| *Archidendron pauciflorum* | 0.47 c | 0.63 b | 1.73 c | 6.88 c | 9.90 d | 3.60 |
| *Leucaena leucocephala* | 4.67 a | 9.02 a | 12.32 b | 29.32 b | n.s | 11.16 |
| *Parkia speciosa* | 0.48 c | 0.57 b | 1.27 c | 2.33 d | n.s | 0.87 |
| *Acacia auriculiformis* | 2.55 b | n.s | n.s | n.s | 20.43 c | 3.98 |
| **DF11** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | 61.92 a | 82.00 a | 81.20 |
| *Falcataria moluccana* | n.s | 12.50 a | 27.47 a | 54.93 a | 73.00 a | 47.93 |
| *Archidendron pauciflorum* | 0.35 c | 0.35 b | 3.37 c | 4.42 c | 6.92 e | 2.19 |
| *Leucaena leucocephala* | 5.58 a | 11.17 a | 16.53 b | 36.63 b | 54.27 b | 19.69 |
| *Parkia speciosa* | 0.25 c | 0.48 b | 1.58 c | 11.97 d | 12.27 d | 5.79 |
| *Acacia auriculiformis* | 2.83 b | n.s | n.s | n.s | 21.28 c | 5.05 |

n.s: No sample, cfu: colonyforming unit

[a] *F. oxysporum* population calculated at the end of the experiment (21 days after inoculation).

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

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**Table 3.** *Fusarium oxysporum* population average and diseases index of plant

| Plant species | Population average (×$10^4$ CFU/g fresh weight) [a] | | | Disease index [b] | | |
|---|---|---|---|---|---|---|
| | AF01 [c] | BF05 | DF11 | AF01 | BF05 | DF11 |
| *Acacia crassicarpa* | 85.13 | 92.61 | 81.20 | 4.00 | 3.48 | 3.96 |
| *Falcataria moluccana* | 76.50 | 43.85 | 47.93 | 3.44 | 3.04 | 2.80 |
| *Archidendron pauciflorum* | 5.06 | 3.60 | 2.19 | 1.96 | 1.88 | 1.40 |
| *Leucaena leucocephala* | 22.13 | 11.16 | 19.69 | 1.52 | 1.56 | 1.68 |
| *Parkia speciosa* | 2.16 | 0.87 | 5.79 | 1.80 | 1.04 | 2.16 |
| *Acacia auriculiformis* | 4.65 | 3.98 | 5.05 | 0.36 | 0.40 | 0.60 |

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4 : A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.
[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.
[c] *F. oxysporum* isolates.

## Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala,* and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotype showed a population level of 15.4 × $10^5$ CFU $g^{-1}$. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslim et al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the induction of cell

wall-associated phenolic compounds. Jiménez-Fernández et al. (2013) also reported that *Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proven to be an alternative host of *F. oxysporum*. Whereas *L. leucocephala*, *A. pauciflorum*, *P. speciosa*, and *A. auriculiformis* had potential as alternative hosts. Many plants of fabaceae family was attacked by formae specialis *F. oxysporum,* such as *Vigna angularis* (*F. oxysporum* f. sp. *adzukicola*), *Cicer arietinum, Cicer* spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp. *koae*), *Lens culinaris, L. esculenta* (*F. oxysporum* f. sp. *lentis*), *Medicago sativa* (*F. oxysporum* f. sp. *medicaginis*), *Phaseolus vulgaris, P. coccineus* (*F. oxysporum* f. sp. *phaseoli*), *Pisum sativum, Cicer arietinum* (*F. oxysporum* f. sp. *pisi*) (Edel-Hermann and Lecomte 2019). However, in this study, *F. oxysporum* isolated from *A. mangium* has a wide host range from fabaceae family; therefore, it is not classified as formae specialis.

In conclusion, *F. oxysporum* isolated from *A. mangium* causes infection in several types of forest and industrial plants. Since it has a wide host range, it is not classified as part of the formae specialis group.

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# Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*

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**Abstract.** *Soleha S, Muslim A, Suwandi S, Kadir S, Pratama R. 2021. Host range studies of* Fusarium oxysporum*, causal agent of seedling wilt disease of* Acacia mangium*. Biodiversitas 23: xxxx. Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-□) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

**Keyword:** *Acacia mangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

## INTRODUCTION

*Acacia mangium* (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asif et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusarium oxysporum* is an important pathogenic fungus that causes wilt disease in different plants all over the world. Soleha et al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017; Muslim et al. 2019). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A. mangium,* which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana,* and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum,* causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

## MATERIALS AND METHOD

### Fungal isolates

Three pathogenic isolates of *F. oxysporum* (AF01, BF05, and DF11) were selected, which were differentiated according to their *tef* 1-α sequence (Figure 1). Isolates were cultured on PDB liquid medium (potato dextrose broth) and incubated at 26-28 °C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered using two layers of sterile gauze to separate the conidia and hyphae. The conidial concentration was determined using a hemocytometer and then adjusted to a concentration of $10^6$ $ml^{-1}$ for pathogenicity test.

### Plant material

The plants used were members of the fabaceae family, namely *A. crassicarpa, A. auriculiformis*, *F. moluccana*, *A. pauciflorum*, *P. speciosa*, and *L. leucocephala*, which were one month old. The seedlings were obtained from the Forest Crops Research Institute, South Sumatra. Seedlings were transferred in a mixed medium with cocopeat (1:1) using a plastic pot of 10 cm diameter and 10 cm height, and then placed in a shade house.

### Pathogenicity test

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension ($10^6$ conidia $ml^{-1}$) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity was calculated using the method of Muslim et al. (2003a) and the disease index (DI) was classified into following grades, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling (Bertetti et al. 2018). Furthermore, plant responses were grouped as, R = resistant (DI=0), MR = moderately resistant/tolerance (DI = <1), MS = moderately susceptible (DI = 1.0–2.0), S = susceptible (DI = 2.1–3.0) and HS = highly susceptible (DI = 3.1–4.0). The development of disease was observed 1–21 days after inoculation.

### *Fusarium oxysporum* population

The population of *F. oxysporum* in the roots was calculated at the end of the experiment using the method of (Muslim et al. 2003b; Li et al. 2008; Horinouchi et al. 2011) with modifications to the surface sterilization of samples. Then the plants were grouped according to severity (disease score) and washed separately under running water to remove soil residues. After that, all plants in each score were surface sterilised using 1% sodium hypochlorite for 15 minutes, then rinsed three times with distilled water. The samples and water (1:100 w/v) were homogenised using blender at 8000 rpm for 10 minutes. Then they were filtered using two layers of sterile gauze and diluted 10 to 1000 times. The suspension was spread on Peptone PCNB agar Media (PPA/Nash Snyder Medium) (Leslie and Summerell 2006) in triplicate (five Petri dishes per replication) and incubated in dark for seven days at room temperature. The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the level of diseases severity.

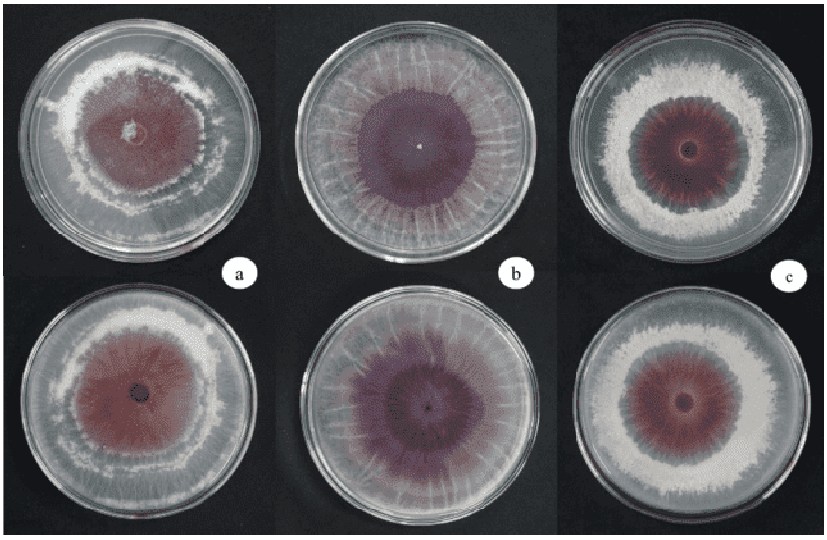


**Figure 1.** *F. oxysporum* isolates on PDA medium. (a) AF01, (b) BF05, and (c) DF11. First line: front view; second line: reverse view.

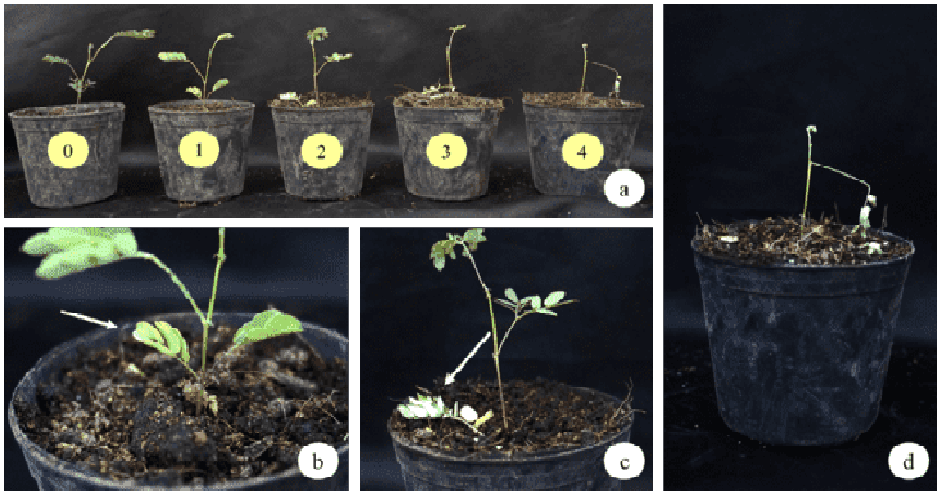


**Figure 2.** Disease index of *Acacia crassicarpa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

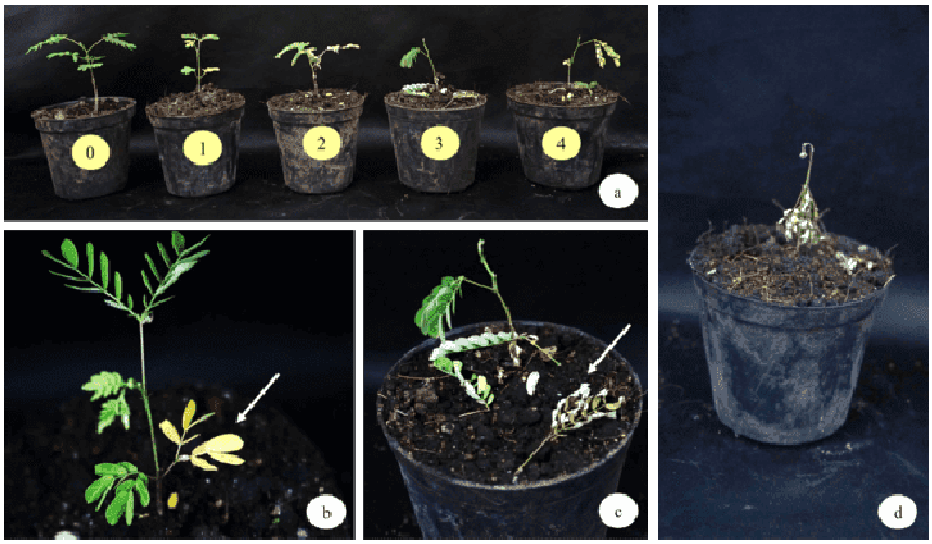


**Figure 3.** Disease index on *Falcataria moluccana*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

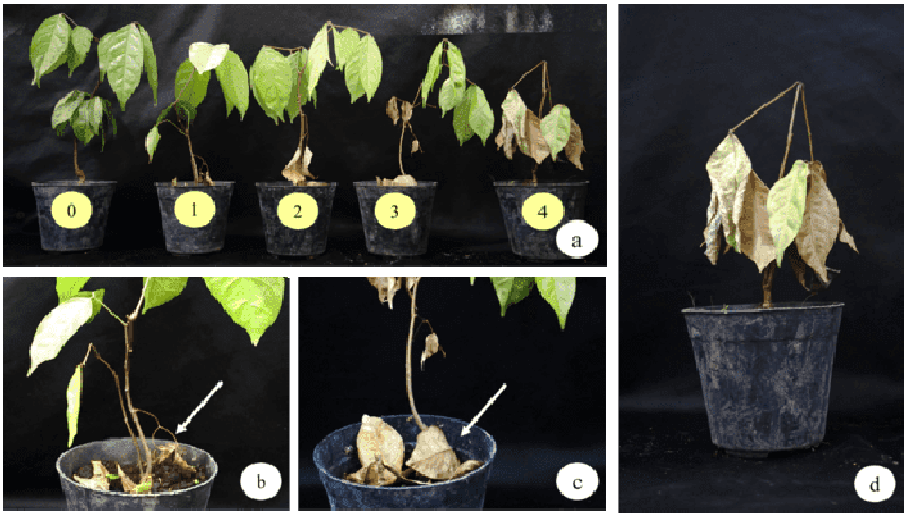


**Figure 4.** Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

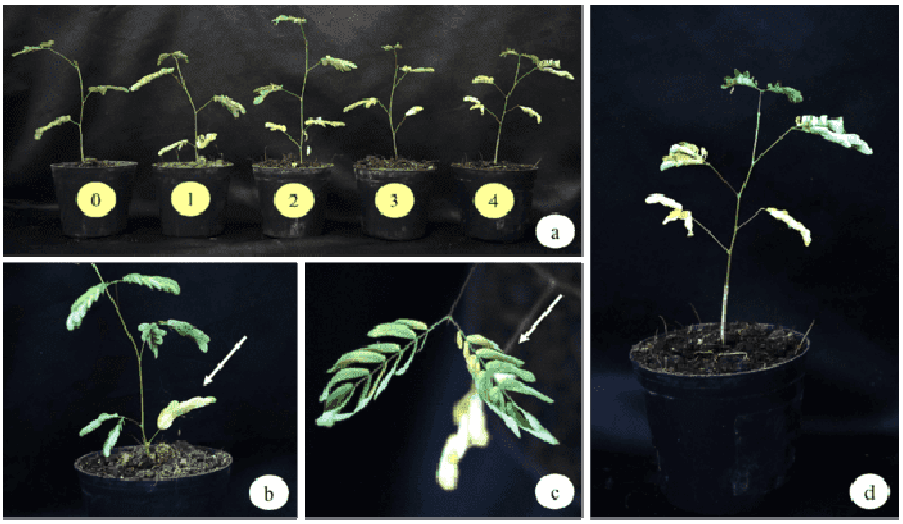


**Figure 5.** Disease index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

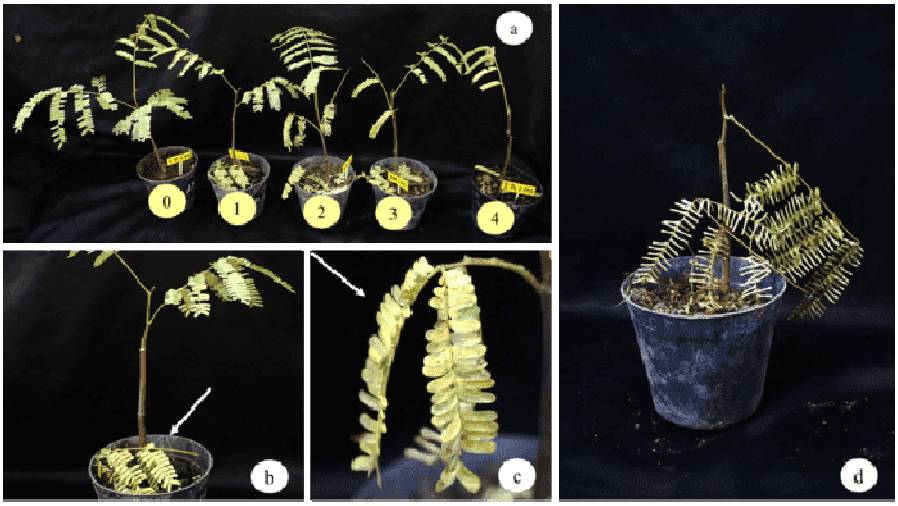


**Figure 6.** Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant


**Figure 7.** Disease index on *Acacia auriculiformis*, from left: healthy plant to wilted and dead plant (score 0–4)

## RESULTS AND DISCUSSION

### Pathogenicity test

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing the leaf colour, while control plants did not show any symptoms (Figures 2-7).

Disease severity was significantly higher than controls. *A. crassicarpa* and *F. moluccana* were most severely affected with an average score of 4.00 and 3.44, respectively. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were showed moderate disease severity i.e. 1.96, 1.68, and 1.80, respectively, whereas *A. auriculiformis* had the lowest (0.36) disease severity (Table 1). Based on the disease score, host plants were classified into three groups: i) highly susceptible (*A. crassicarpa* and *F. moluccana*), ii) moderately susceptible (*A. pauciflorum P. speciosa*, and *L. leucocephala*), and iii) moderate resistance/tolerance (*A. auriculiformis*). Result exhibited that there was no significant difference between the disease severity in the same host that had been inoculated with different isolates (Table 1).

**Table 1.** Disease severity and host responses to *Fusarium oxysporum* isolated from *Acacia mangium*

| Plant species | Isolates [a)] | | | | | |
|---|---|---|---|---|---|---|
| | AF01 [b)] | Response [c)] | BF05 | Response | DF11 | Response |
| *Acacia crassicarpa* | 4.00 a | HS [c)] | 3.48 a | HS | 3.96 a | HS |
| *Falcataria moluccana* | 3.44 ab | HS | 3.04 a | HS | 2.80 ab | S |
| *Archidendron pauciflorum* | 1.96 bc | MS | 1.88 b | MS | 1.40 cd | MS |
| *Leucaena leucocephala* | 1.52 c | MS | 1.56 b | MS | 1.68 bc | MS |
| *Parkia speciosa* | 1.80 c | MS | 1.04 bc | MS | 2.16 bc | S |
| *Acacia auriculiformis* | 0.36 d | MR | 0.40 c | MR | 0.60 d | MR |

Values followed by the same letter in each row are not significant.

[a] DI 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.

[b)] *F. oxysporum* isolates.

[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI = 1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

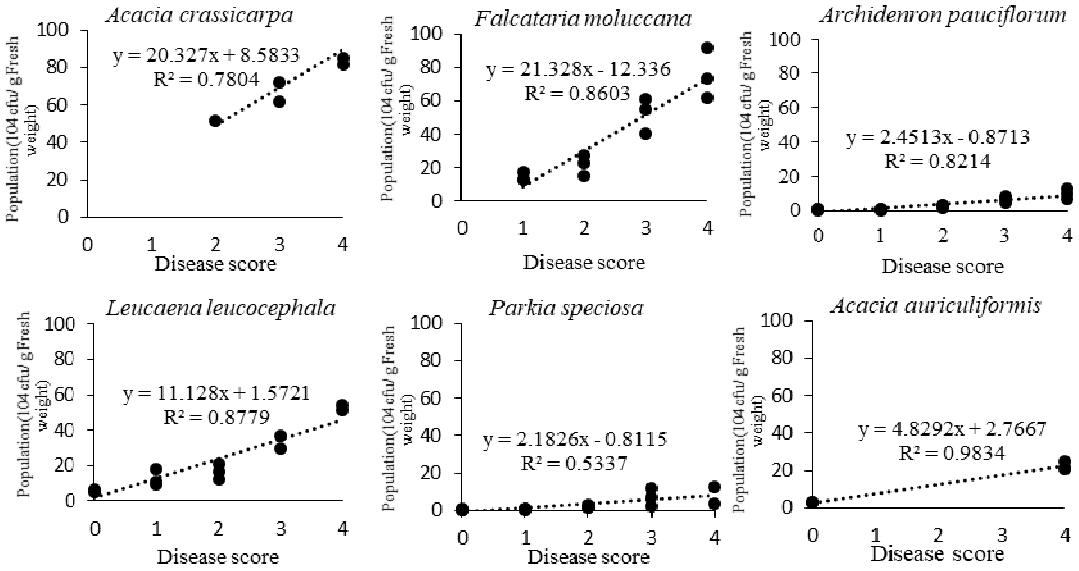


**Figure 8.** Regression analysis of disease score rate and *F. oxysporum* population

**Table 2.** *Fusarium oxysporum* population on root in each disease index

| Plant species | Population of *Fusarium oxysporum* (×10$^4$ CFU/g fresh weight) [a] | | | | | Average [c] |
|---|---|---|---|---|---|---|
| | 0 [b] | 1 | 2 | 3 | 4 | |
| **AF01** [d] | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | n.s | 85.13 a[e] | 85.13 |
| *Falcataria moluccana* | n.s | 17.77 a | 22.77 a | 60.98 a | 91.87 a | 76.50 |
| *Archidendron pauciflorum* | 0.45 b | 1.10 b | 3.22 b | 8.15 b | 12.53 cd | 5.06 |
| *Leucaena leucocephala* | 6.17 a | 18.10 a | 20.93 a | n.s | 51.67 b | 22.13 |
| *Parkia speciosa* | 0.32 b | 0.45 b | 2.58 b | 7.27 b | 3.57 d | 2.16 |
| *Acacia auriculiformis* | 2.92 a | n.s | n.s | n.s | 24.53 c | 4.65 |
| **BF05** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | 51.80 a | 72.08 a | 105.10 a | 92.61 |
| *Falcataria moluccana* | n.s | 13.22 a | 15.32 b | 40.33 b | 61.67 b | 43.85 |
| *Archidendron pauciflorum* | 0.47 c | 0.63 b | 1.73 c | 6.88 c | 9.90 d | 3.60 |
| *Leucaena leucocephala* | 4.67 a | 9.02 a | 12.32 b | 29.32 b | n.s | 11.16 |
| *Parkia speciosa* | 0.48 c | 0.57 b | 1.27 c | 2.33 d | n.s | 0.87 |
| *Acacia auriculiformis* | 2.55 b | n.s | n.s | n.s | 20.43 c | 3.98 |
| **DF11** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | 61.92 a | 82.00 a | 81.20 |
| *Falcataria moluccana* | n.s | 12.50 a | 27.47 a | 54.93 a | 73.00 a | 47.93 |
| *Archidendron pauciflorum* | 0.35 c | 0.35 b | 3.37 c | 4.42 c | 6.92 e | 2.19 |
| *Leucaena leucocephala* | 5.58 a | 11.17 a | 16.53 b | 36.63 b | 54.27 b | 19.69 |
| *Parkia speciosa* | 0.25 c | 0.48 b | 1.58 c | 11.97 d | 12.27 d | 5.79 |
| *Acacia auriculiformis* | 2.83 b | n.s | n.s | n.s | 21.28 c | 5.05 |

n.s: No sample, cfu: colonyforming unit

[a] *F. oxysporum* population calculated at the end of the experiment (21 days after inoculation).

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

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**Table 3.** *Fusarium oxysporum* population average and diseases index of plant

| Plant species | Population average (×$10^4$ CFU/g fresh weight) [a] | | | Disease index [b] | | |
|---|---|---|---|---|---|---|
| | AF01 [c] | BF05 | DF11 | AF01 | BF05 | DF11 |
| *Acacia crassicarpa* | 85.13 | 92.61 | 81.20 | 4.00 | 3.48 | 3.96 |
| *Falcataria moluccana* | 76.50 | 43.85 | 47.93 | 3.44 | 3.04 | 2.80 |
| *Archidendron pauciflorum* | 5.06 | 3.60 | 2.19 | 1.96 | 1.88 | 1.40 |
| *Leucaena leucocephala* | 22.13 | 11.16 | 19.69 | 1.52 | 1.56 | 1.68 |
| *Parkia speciosa* | 2.16 | 0.87 | 5.79 | 1.80 | 1.04 | 2.16 |
| *Acacia auriculiformis* | 4.65 | 3.98 | 5.05 | 0.36 | 0.40 | 0.60 |

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4 : A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.
[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.
[c] *F. oxysporum* isolates.

## Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala,* and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotype showed a population level of 15.4 × $10^5$ CFU $g^{-1}$. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslim et al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the induction of cell

wall-associated phenolic compounds. Jiménez-Fernández et al. (2013) also reported that *Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proven to be an alternative host of *F. oxysporum*. Whereas *L. leucocephala*, *A. pauciflorum*, *P. speciosa*, and *A. auriculiformis* had potential as alternative hosts. Many plants of fabaceae family was attacked by formae specialis *F. oxysporum,* such as *Vigna angularis* (*F. oxysporum* f. sp. *adzukicola*), *Cicer arietinum, Cicer* spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp. *koae*), *Lens culinaris, L. esculenta* (*F. oxysporum* f. sp. *lentis*), *Medicago sativa* (*F. oxysporum* f. sp. *medicaginis*), *Phaseolus vulgaris, P. coccineus* (*F. oxysporum* f. sp. *phaseoli*), *Pisum sativum, Cicer arietinum* (*F. oxysporum* f. sp. *pisi*) (Edel-Hermann and Lecomte 2019). However, in this study, *F. oxysporum* isolated from *A. mangium* has a wide host range from fabaceae family; therefore, it is not classified as formae specialis.

In conclusion, *F. oxysporum* isolated from *A. mangium* causes infection in several types of forest and industrial plants. Since it has a wide host range, it is not classified as part of the formae specialis group.

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# Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*

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**Abstract.** *Soleha S, Muslim A, Suwandi S, Kadir S, Pratama R. 2021. Host range studies of* Fusarium oxysporum*, causal agent of seedling wilt disease of* Acacia mangium*. Biodiversitas 23: xxxx. Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-□) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

**Keyword:** *Acacia mangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

## INTRODUCTION

*Acacia mangium* (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asif et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusarium oxysporum* is an important pathogenic fungus that causes wilt disease in different plants all over the world. Soleha et al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017; Muslim et al. 2019). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A. mangium,* which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana,* and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum,* causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

## MATERIALS AND METHOD

### Fungal isolates

Three pathogenic isolates of *F. oxysporum* (AF01, BF05, and DF11) were selected, which were differentiated according to their *tef* 1-α sequence (Figure 1). Isolates were cultured on PDB liquid medium (potato dextrose broth) and incubated at 26-28 °C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered using two layers of sterile gauze to separate the conidia and hyphae. The conidial concentration was determined using a hemocytometer and then adjusted to a concentration of $10^6$ $ml^{-1}$ for pathogenicity test.

### Plant material

The plants used were members of the fabaceae family, namely *A. crassicarpa, A. auriculiformis*, *F. moluccana*, *A. pauciflorum*, *P. speciosa*, and *L. leucocephala*, which were one month old. The seedlings were obtained from the Forest Crops Research Institute, South Sumatra. Seedlings were transferred in a mixed medium with cocopeat (1:1) using a plastic pot of 10 cm diameter and 10 cm height, and then placed in a shade house.

### Pathogenicity test

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension ($10^6$ conidia $ml^{-1}$) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity was calculated using the method of Muslim et al. (2003a) and the disease index (DI) was classified into following grades, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling (Bertetti et al. 2018). Furthermore, plant responses were grouped as, R = resistant (DI=0), MR = moderately resistant/tolerance (DI = <1), MS = moderately susceptible (DI = 1.0–2.0), S = susceptible (DI = 2.1–3.0) and HS = highly susceptible (DI = 3.1–4.0). The development of disease was observed 1–21 days after inoculation.

### *Fusarium oxysporum* population

The population of *F. oxysporum* in the roots was calculated at the end of the experiment using the method of (Muslim et al. 2003b; Li et al. 2008; Horinouchi et al. 2011) with modifications to the surface sterilization of samples. Then the plants were grouped according to severity (disease score) and washed separately under running water to remove soil residues. After that, all plants in each score were surface sterilised using 1% sodium hypochlorite for 15 minutes, then rinsed three times with distilled water. The samples and water (1:100 w/v) were homogenised using blender at 8000 rpm for 10 minutes. Then they were filtered using two layers of sterile gauze and diluted 10 to 1000 times. The suspension was spread on Peptone PCNB agar Media (PPA/Nash Snyder Medium) (Leslie and Summerell 2006) in triplicate (five Petri dishes per replication) and incubated in dark for seven days at room temperature. The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the level of diseases severity.

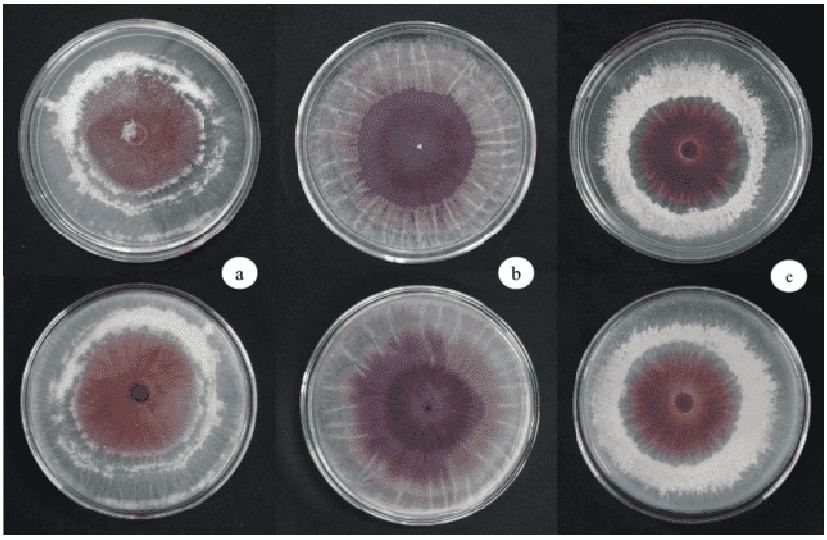


**Figure 1.** *F. oxysporum* isolates on PDA medium. (a) AF01, (b) BF05, and (c) DF11. First line: front view; second line: reverse view.

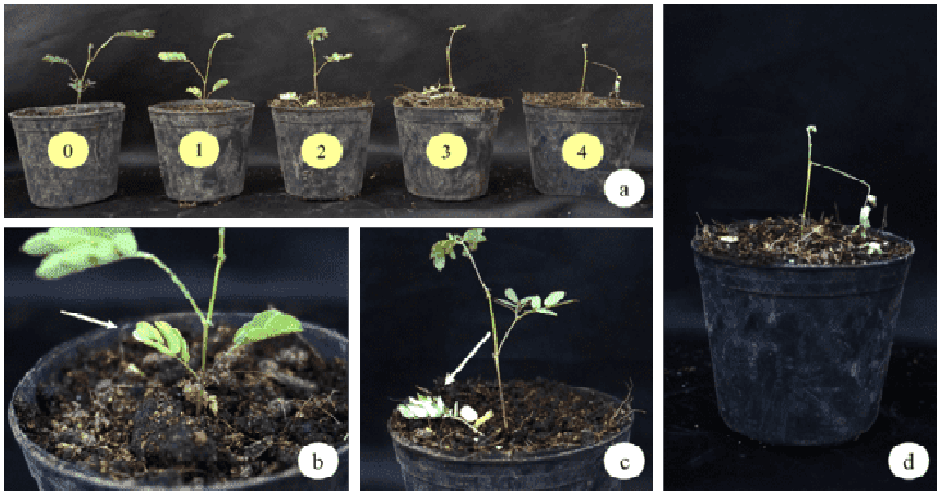


**Figure 2.** Disease index of *Acacia crassicarpa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

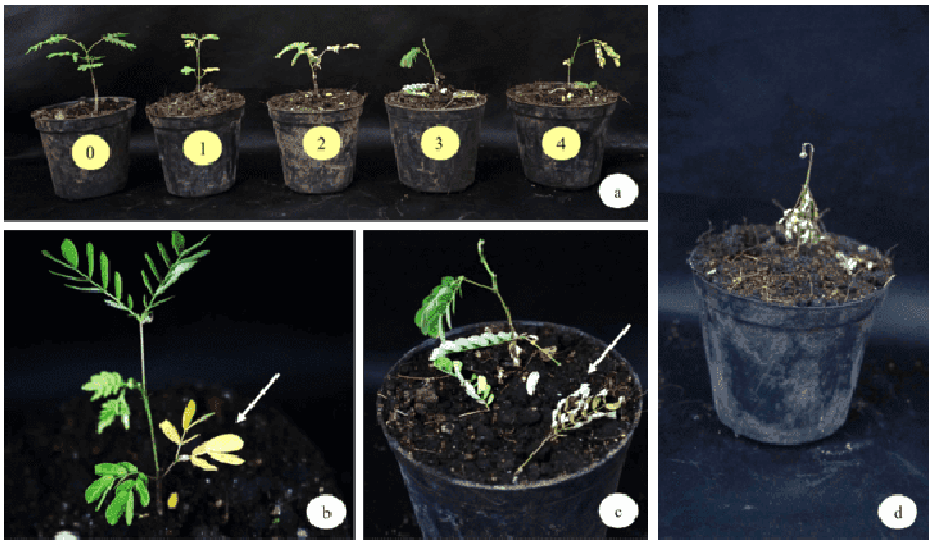


**Figure 3.** Disease index on *Falcataria moluccana*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

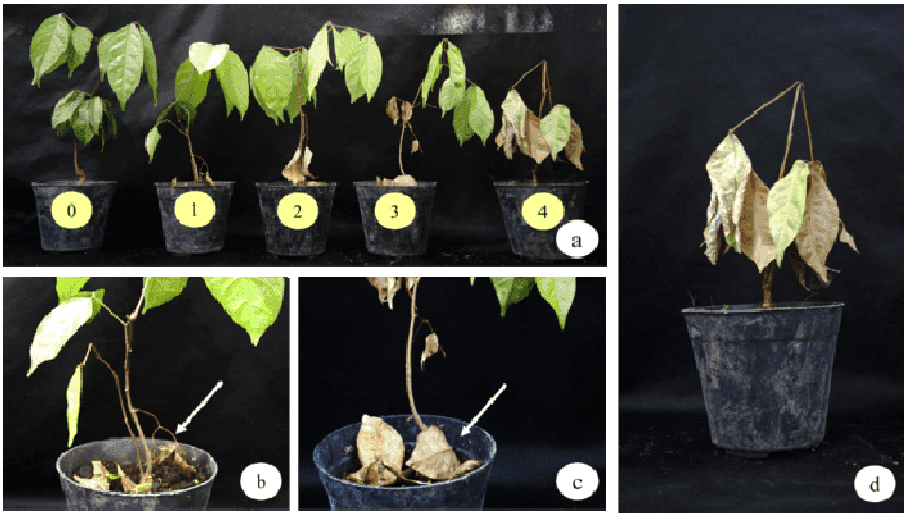


**Figure 4.** Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

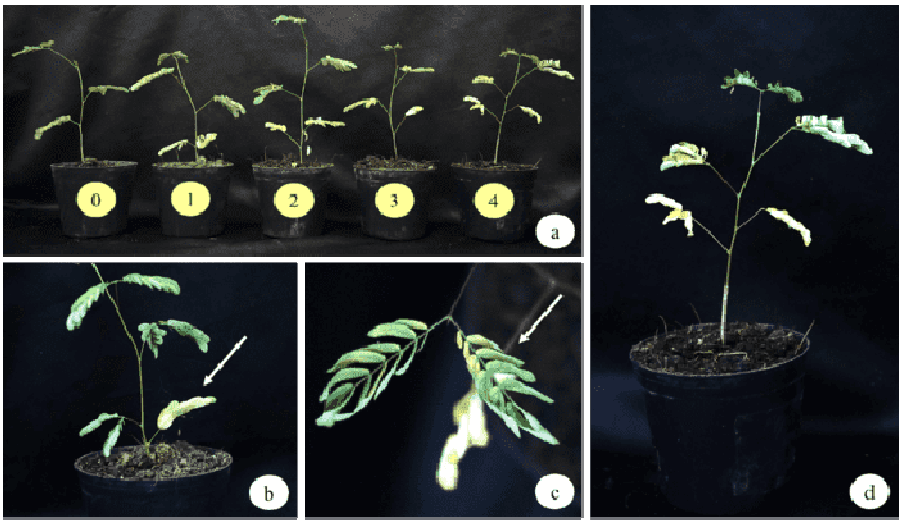


**Figure 5.** Disease index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

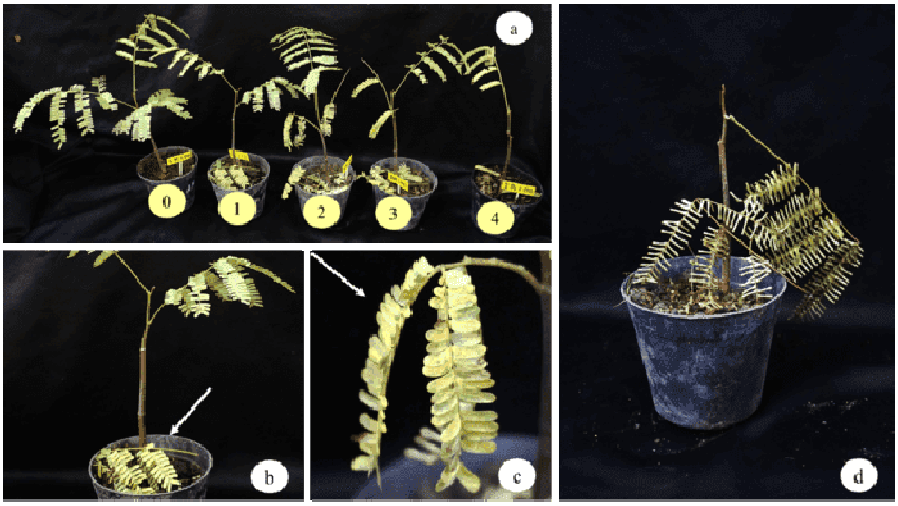


**Figure 6.** Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant


**Figure 7.** Disease index on *Acacia auriculiformis*, from left: healthy plant to wilted and dead plant (score 0–4)

## RESULTS AND DISCUSSION

### Pathogenicity test

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing the leaf colour, while control plants did not show any symptoms (Figures 2-7).

Disease severity was significantly higher than controls. *A. crassicarpa* and *F. moluccana* were most severely affected with an average score of 4.00 and 3.44, respectively. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were showed moderate disease severity i.e. 1.96, 1.68, and 1.80, respectively, whereas *A. auriculiformis* had the lowest (0.36) disease severity (Table 1). Based on the disease score, host plants were classified into three groups: i) highly susceptible (*A. crassicarpa* and *F. moluccana*), ii) moderately susceptible (*A. pauciflorum P. speciosa*, and *L. leucocephala*), and iii) moderate resistance/tolerance (*A. auriculiformis*). Result exhibited that there was no significant difference between the disease severity in the same host that had been inoculated with different isolates (Table 1).

### *Fusarium oxysporum* population

The total population of *F. oxysporum* on the roots was determined by calculating the CFU for each category of damage. For DI 4, *A. crassicarpa* and *F. moluccana* showed a significantly higher population (82.00–105.10 × $10^4$ CFU $g^{-1}$ fresh weight) than other plants. The lowest population was recorded in *P. speciosa* and *A. pauciflorum* (3.57–12.27 × $10^4$ CFU $g^{-1}$ fresh weight). This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. auriculiformis* for DI 2 and 3. In DI 1, the highest population was recorded in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample for DI 1. In inoculated plants with DI 0, the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana* (Table 2 &Table 3).

The regression analysis results showed that all plants except *P. speciosa* had a linear relationship pattern between the increase in disease score and population. The pathogenic population on *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, as indicated by the magnitude of regression gradient coefficient (m=20.3–21.3). However, moderate increase was observed in *L. leucocephala* (m=11.2) (m=11.2) and very slow in *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Table 3 showed that isolates were different in *tef1-α*, but the population and DI patterns were similar for each test plant. The correlation between the population of pathogen ($g^{-1}$ fresh weight) and the level of DI were described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

**Table 1.** Disease severity and host responses to *Fusarium oxysporum* isolated from *Acacia mangium*

| Plant species | Isolates [a)] | | | | | |
|---|---|---|---|---|---|---|
| | AF01 [b)] | Response [c)] | BF05 | Response | DF11 | Response |
| *Acacia crassicarpa* | 4.00 a | HS [c)] | 3.48 a | HS | 3.96 a | HS |
| *Falcataria moluccana* | 3.44 ab | HS | 3.04 a | HS | 2.80 ab | S |
| *Archidendron pauciflorum* | 1.96 bc | MS | 1.88 b | MS | 1.40 cd | MS |
| *Leucaena leucocephala* | 1.52 c | MS | 1.56 b | MS | 1.68 bc | MS |
| *Parkia speciosa* | 1.80 c | MS | 1.04 bc | MS | 2.16 bc | S |
| *Acacia auriculiformis* | 0.36 d | MR | 0.40 c | MR | 0.60 d | MR |

Values followed by the same letter in each row are not significant.

[a] DI 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.

[b)] *F. oxysporum* isolates.

[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI = 1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

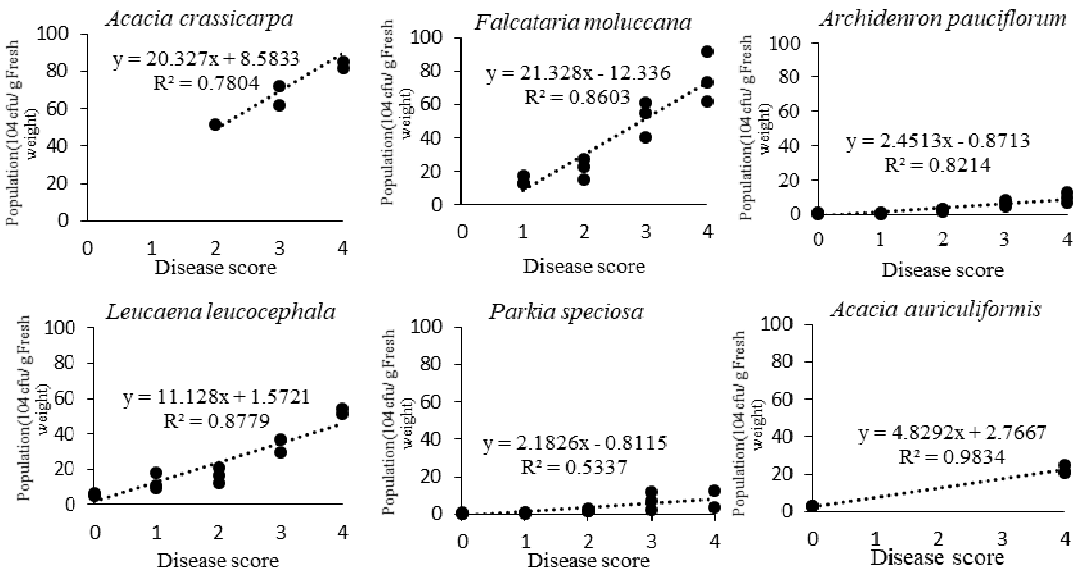


**Figure 8.** Regression analysis of disease score rate and *F. oxysporum* population

**Table 2.** *Fusarium oxysporum* population on root in each disease index

| Plant species | Population of *Fusarium oxysporum* (×10$^4$ CFU/g fresh weight) [a] | | | | | Average [c] |
|---|---|---|---|---|---|---|
| | 0 [b] | 1 | 2 | 3 | 4 | |
| **AF01** [d] | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | n.s | 85.13 a[e] | 85.13 |
| *Falcataria moluccana* | n.s | 17.77 a | 22.77 a | 60.98 a | 91.87 a | 76.50 |
| *Archidendron pauciflorum* | 0.45 b | 1.10 b | 3.22 b | 8.15 b | 12.53 cd | 5.06 |
| *Leucaena leucocephala* | 6.17 a | 18.10 a | 20.93 a | n.s | 51.67 b | 22.13 |
| *Parkia speciosa* | 0.32 b | 0.45 b | 2.58 b | 7.27 b | 3.57 d | 2.16 |
| *Acacia auriculiformis* | 2.92 a | n.s | n.s | n.s | 24.53 c | 4.65 |
| **BF05** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | 51.80 a | 72.08 a | 105.10 a | 92.61 |
| *Falcataria moluccana* | n.s | 13.22 a | 15.32 b | 40.33 b | 61.67 b | 43.85 |
| *Archidendron pauciflorum* | 0.47 c | 0.63 b | 1.73 c | 6.88 c | 9.90 d | 3.60 |
| *Leucaena leucocephala* | 4.67 a | 9.02 a | 12.32 b | 29.32 b | n.s | 11.16 |
| *Parkia speciosa* | 0.48 c | 0.57 b | 1.27 c | 2.33 d | n.s | 0.87 |
| *Acacia auriculiformis* | 2.55 b | n.s | n.s | n.s | 20.43 c | 3.98 |
| **DF11** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | 61.92 a | 82.00 a | 81.20 |
| *Falcataria moluccana* | n.s | 12.50 a | 27.47 a | 54.93 a | 73.00 a | 47.93 |
| *Archidendron pauciflorum* | 0.35 c | 0.35 b | 3.37 c | 4.42 c | 6.92 e | 2.19 |
| *Leucaena leucocephala* | 5.58 a | 11.17 a | 16.53 b | 36.63 b | 54.27 b | 19.69 |
| *Parkia speciosa* | 0.25 c | 0.48 b | 1.58 c | 11.97 d | 12.27 d | 5.79 |
| *Acacia auriculiformis* | 2.83 b | n.s | n.s | n.s | 21.28 c | 5.05 |

n.s: No sample, cfu: colonyforming unit

[a] *F. oxysporum* population calculated at the end of the experiment (21 days after inoculation).

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

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**Table 3.** *Fusarium oxysporum* population average and diseases index of plant

| Plant species | Population average (×$10^4$ CFU/g fresh weight) [a] | | | Disease index [b] | | |
|---|---|---|---|---|---|---|
| | AF01 [c] | BF05 | DF11 | AF01 | BF05 | DF11 |
| *Acacia crassicarpa* | 85.13 | 92.61 | 81.20 | 4.00 | 3.48 | 3.96 |
| *Falcataria moluccana* | 76.50 | 43.85 | 47.93 | 3.44 | 3.04 | 2.80 |
| *Archidendron pauciflorum* | 5.06 | 3.60 | 2.19 | 1.96 | 1.88 | 1.40 |
| *Leucaena leucocephala* | 22.13 | 11.16 | 19.69 | 1.52 | 1.56 | 1.68 |
| *Parkia speciosa* | 2.16 | 0.87 | 5.79 | 1.80 | 1.04 | 2.16 |
| *Acacia auriculiformis* | 4.65 | 3.98 | 5.05 | 0.36 | 0.40 | 0.60 |

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4 : A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.
[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.
[c] *F. oxysporum* isolates.

## Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala,* and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose,* and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotype showed a population level of 15.4 × $10^5$ CFU $g^{-1}$. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslim et al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the induction of cell

wall-associated phenolic compounds. Jiménez-Fernández et al. (2013) also reported that *Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proven to be an alternative host of *F. oxysporum*. Whereas *L. leucocephala*, *A. pauciflorum*, *P. speciosa*, and *A. auriculiformis* had potential as alternative hosts. Many plants of fabaceae family was attacked by formae specialis *F. oxysporum,* such as *Vigna angularis* (*F. oxysporum* f. sp. *adzukicola*), *Cicer arietinum, Cicer* spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp. *koae*), *Lens culinaris, L. esculenta* (*F. oxysporum* f. sp. *lentis*), *Medicago sativa* (*F. oxysporum* f. sp. *medicaginis*), *Phaseolus vulgaris, P. coccineus* (*F. oxysporum* f. sp. *phaseoli*), *Pisum sativum, Cicer arietinum* (*F. oxysporum* f. sp. *pisi*) (Edel-Hermann and Lecomte 2019). However, in this study, *F. oxysporum* isolated from *A. mangium* has a wide host range from fabaceae family; therefore, it is not classified as formae specialis.

In conclusion, *F. oxysporum* isolated from *A. mangium* causes infection in several types of forest and industrial plants. Since it has a wide host range, it is not classified as part of the formae specialis group.

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The editing of your submission, "Host range studies of Fusarium oxysporum, causal agent of seedling wilt disease of Acacia mangium," is complete. We are now sending it to production.

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# Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*

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**Abstract.** *Soleha S, Muslim A, Suwandi S, Kadir S, Pratama R. 2021. Host range studies of* Fusarium oxysporum*, causal agent of seedling wilt disease of* Acacia mangium*. Biodiversitas 23: 25-32. Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra, Indonesia. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forests and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-α) sequences were tested for pathogenicity on different Fabaceae family plants and the growth of population was also observed. The results showed that all three isolates were able to infect all the tested plants with different reactions to wilt disease. *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the Fabaceae family.

**Keywords:** *Acacia mangium*, Fabaceae, *Fusarium oxysporum*, host range, seedling wilt

## INTRODUCTION

*Acacia mangium* (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asif et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusarium oxysporum* is an important pathogenic fungus that causes wilt disease in different plants all over the world. Soleha et al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017; Muslim et al. 2019). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013), and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A. mangium*, which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana*, and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum*, causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

## MATERIALS AND METHODS

### Fungal isolates

Three pathogenic isolates of *F. oxysporum* (AF01, BF05, and DF11) were selected, which were differentiated according to their *tef* 1-α sequence (Figure 1). Isolates were cultured on PDB liquid medium (potato dextrose broth) and incubated at 26-28°C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered using two layers of sterile gauze to separate the conidia and hyphae. The conidial concentration was determined using a hemocytometer and then adjusted to a concentration of $10^6$ $ml^{-1}$ for pathogenicity test.

### Plant material

The plants used were members of the Fabaceae family, namely *A. crassicarpa*, *A. auriculiformis*, *F. moluccana*, *A. pauciflorum*, *P. speciosa*, and *L. leucocephala*, which were one month old. The seedlings were obtained from the Forest Crops Research Institute, South Sumatra. Seedlings were transferred in a mixed medium with cocopeat (1:1) using a plastic pot of 10 cm diameter and 10 cm height, and then placed in a shade house.

### Pathogenicity test

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension ($10^6$ conidia $ml^{-1}$) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity was calculated using the method of Muslim et al. (2003a) and the disease index (DI) was classified into following grades, where 0 : no disease/healthy seedling, 1 : yellow leaves, 2 : yellow leaves and slightly wilted, 3 : severe wilt, and 4 : dead seedling (Bertetti et al. 2018). Furthermore, plant responses were grouped as, R : resistant (DI=0), MR : moderately resistant/tolerance (DI = <1), MS : moderately susceptible (DI = 1.0–2.0), S : susceptible (DI = 2.1–3.0) and HS : highly susceptible (DI = 3.1–4.0). The development of disease was observed 1–21 days after inoculation.

### *Fusarium oxysporum* population

The population of *F. oxysporum* in the roots was calculated at the end of the experiment using the method of (Muslim et al. 2003b; Li et al. 2009; Horinouchi et al. 2011) with modifications to the surface sterilization of samples. Then the plants were grouped according to severity (disease score) and washed separately under running water to remove soil residues. After that, all plants in each score were surface sterilized using 1% sodium hypochlorite for 15 minutes, then rinsed three times with distilled water. The samples and water (1:100 w/v) were homogenized using blender at 8000 rpm for 10 minutes. Then they were filtered using two layers of sterile gauze and diluted 10 to 1000 times. The suspension was spread on Peptone PCNB agar Media (PPA/Nash Snyder Medium) (Leslie and Summerell 2006) in triplicate (five Petri dishes per replication) and incubated in dark for seven days at room temperature. The number of colony-forming units (CFU) of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the level of diseases severity.

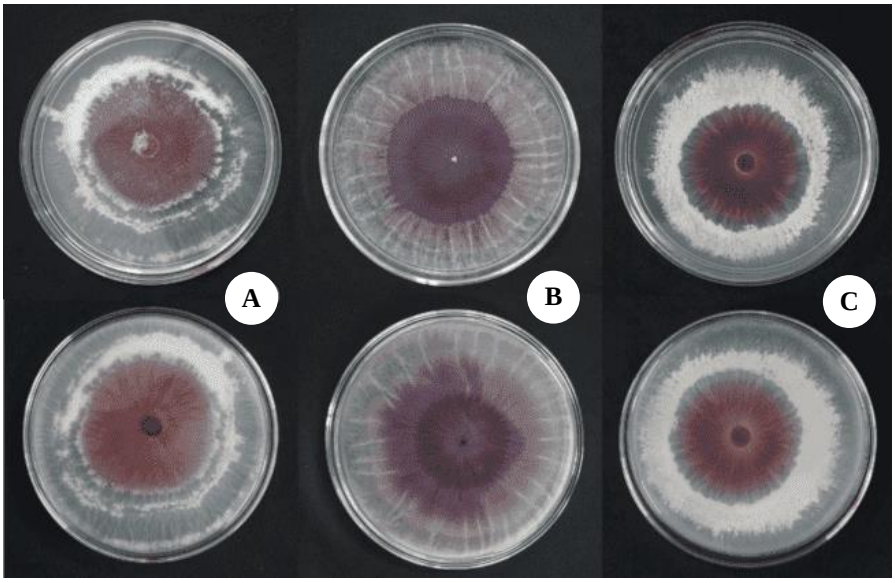


**Figure 1.** *Fusarium oxysporum* isolates on PDA medium. A. AF01, B. BF05, and C. DF11. First line: front view; second line: reverse view

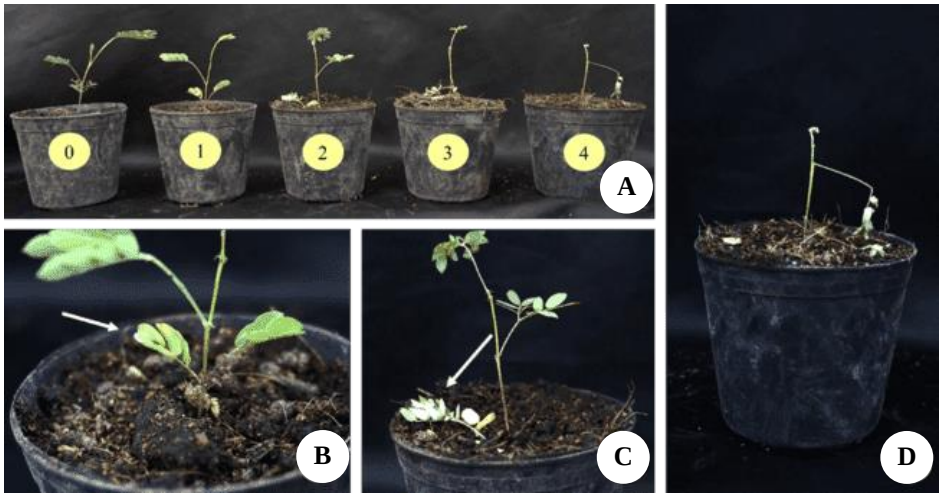


**Figure 2.** Disease index of *Acacia crassicarpa*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: falling leaves; D. Dead plant

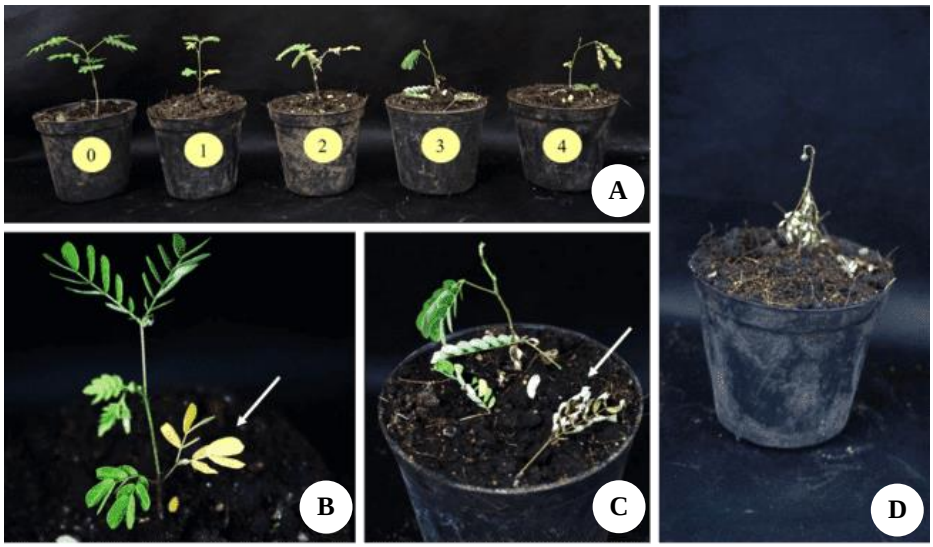


**Figure 3.** Disease index on *Falcataria moluccana*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: curved, dry, and falling leaves; D. Dead plant

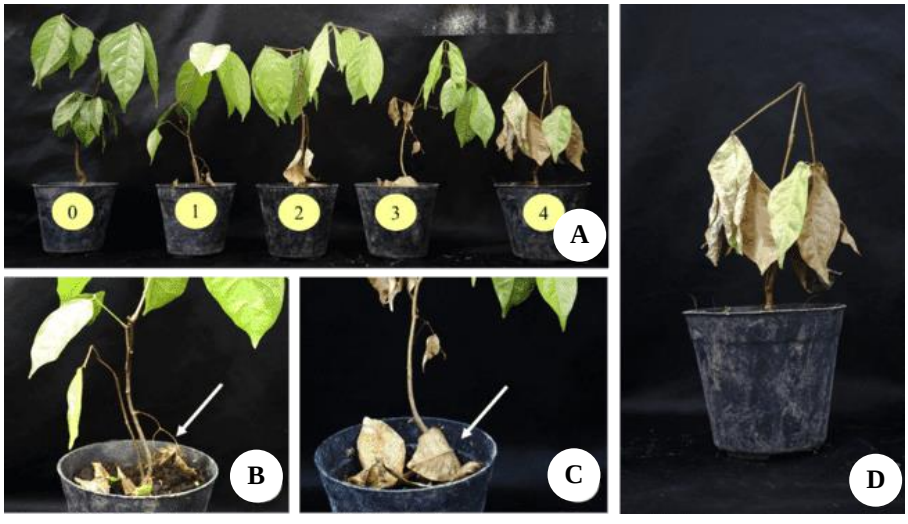


**Figure 4.** Disease index on *Archidendron pauciflorum*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing and dry from oldest leaves; C. Advanced symptoms: falling leaves; D. Dead plant

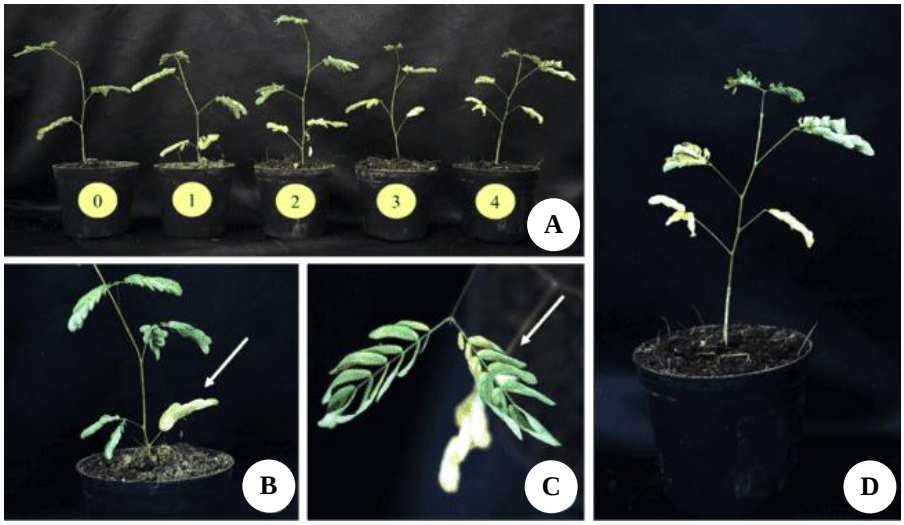


**Figure 5.** Disease index on *Leucaena leucocephala*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: curved leaves; D. Yellowing upward

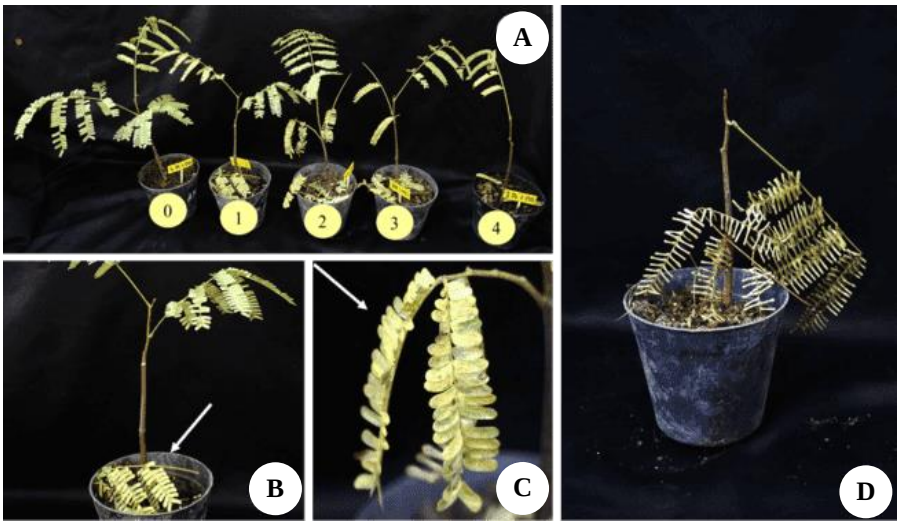


**Figure 6.** Disease index on *Parkia speciosa*. A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing and dry from oldest leaves; C. Advanced symptoms: curved leaves; yellowing, D. Dead plant


**Figure 7.** Disease index on *Acacia auriculiformis*, from left: healthy plant to wilted and dead plant (score 0–4)

## RESULTS AND DISCUSSION

### Pathogenicity test

The results showed that all the six forest plants tested had a similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progresses to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing the leaf color, while control plants did not show any symptoms (Figures 2-7).

Disease severity was significantly higher than controls. *A. crassicarpa* and *F. moluccana* were most severely affected with an average score of 4.00 and 3.44, respectively. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were showed moderate disease severity, i.e. 1.96, 1.68, and 1.80, respectively, whereas *A. auriculiformis* had the lowest (0.36) disease severity (Table 1). Based on the disease score, host plants were classified into three groups: (i) highly susceptible (*A. crassicarpa* and *F. moluccana*), (ii) moderately susceptible (*A. pauciflorum P. speciosa*, and *L. leucocephala*), and (iii) moderate resistance/tolerance (*A. auriculiformis*). Results exhibited that there was no significant difference between the disease severity in the same host that had been inoculated with different isolates (Table 1).

### *Fusarium oxysporum* population

The total population of *F. oxysporum* on the roots was determined by calculating the CFU for each category of damage. For DI 4, *A. crassicarpa* and *F. moluccana* showed a significantly higher population (82.00–105.10 × $10^4$ CFU $g^{-1}$ fresh weight) than other plants. The lowest population was recorded in *P. speciosa* and *A. pauciflorum* (3.57–12.27 × $10^4$ CFU $g^{-1}$ fresh weight). This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. auriculiformis* for DI 2 and 3. In DI 1, the highest population was recorded in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample for DI 1. In inoculated plants with DI 0, the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana* (Table 2 and Table 3).

The regression analysis results showed that all plants except *P. speciosa* had a linear relationship pattern between the increase in disease score and population. The pathogenic population on *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, as indicated by the magnitude of regression gradient coefficient (m=20.3–21.3). However, moderate increase was observed in *L. leucocephala* (m=11.2) (m=11.2) and very slow in *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Table 3 showed that isolates were different in *tef1-α*, but the population and DI patterns were similar for each test plant. The correlation between the population of pathogen ($g^{-1}$ fresh weight) and the level of DI was described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

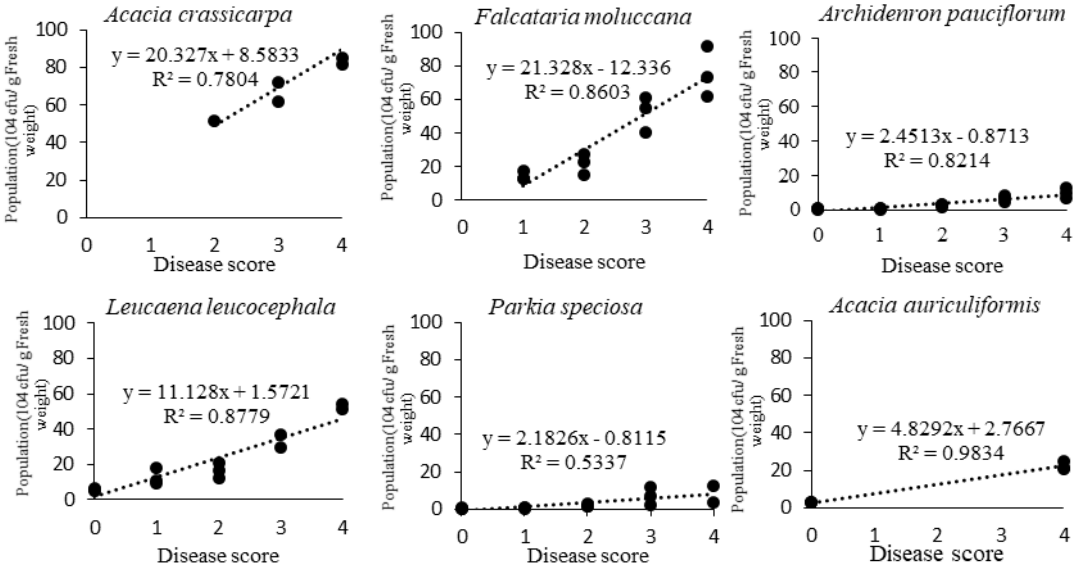


**Figure 8.** Regression analysis of disease score rate and *Fusarium oxysporum* population

**Table 1.** Disease severity and host responses to *Fusarium oxysporum* isolated from *Acacia mangium*

| Plant species | Isolates [a] | | | | | |
|---|---|---|---|---|---|---|
| | AF01 [b] | Response [c] | BF05 | Response | DF11 | Response |
| *Acacia crassicarpa* | 4.00 a | HS [c] | 3.48 a | HS | 3.96 a | HS |
| *Falcataria moluccana* | 3.44 ab | HS | 3.04 a | HS | 2.80 ab | S |
| *Archidendron pauciflorum* | 1.96 bc | MS | 1.88 b | MS | 1.40 cd | MS |
| *Leucaena leucocephala* | 1.52 c | MS | 1.56 b | MS | 1.68 bc | MS |
| *Parkia speciosa* | 1.80 c | MS | 1.04 bc | MS | 2.16 bc | S |
| *Acacia auriculiformis* | 0.36 d | MR | 0.40 c | MR | 0.60 d | MR |

Note: Values followed by the same letter in each row are not significant. [a] DI 0–4, where 0: no disease/healthy seedling, 1: yellow leaves, 2: yellow leaves and slightly wilted, 3: severe wilt, and 4: dead seedling. [b] *F. oxysporum* isolates. [c] Host response grouped as: R: resistant (DI = 0); MR: moderately resistant/tolerance (DI = <1); MS: moderately susceptible (DI = 1.0–2.0); S: susceptible (DI = 2.1–3.0); HS: highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

**Table 2.** *Fusarium oxysporum* population on root in each disease index

| Plant species | Population of *Fusarium oxysporum* (×10$^4$ CFU/g fresh weight) [a] | | | | | Average [c] |
|---|---|---|---|---|---|---|
| | 0 [b] | 1 | 2 | 3 | 4 | |
| **AF01** [d] | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | n.s | 85.13 a[e] | 85.13 |
| *Falcataria moluccana* | n.s | 17.77 a | 22.77 a | 60.98 a | 91.87 a | 76.50 |
| *Archidendron pauciflorum* | 0.45 b | 1.10 b | 3.22 b | 8.15 b | 12.53 cd | 5.06 |
| *Leucaena leucocephala* | 6.17 a | 18.10 a | 20.93 a | n.s | 51.67 b | 22.13 |
| *Parkia speciosa* | 0.32 b | 0.45 b | 2.58 b | 7.27 b | 3.57 d | 2.16 |
| *Acacia auriculiformis* | 2.92 a | n.s | n.s | n.s | 24.53 c | 4.65 |
| **BF05** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | 51.80 a | 72.08 a | 105.10 a | 92.61 |
| *Falcataria moluccana* | n.s | 13.22 a | 15.32 b | 40.33 b | 61.67 b | 43.85 |
| *Archidendron pauciflorum* | 0.47 c | 0.63 b | 1.73 c | 6.88 c | 9.90 d | 3.60 |
| *Leucaena leucocephala* | 4.67 a | 9.02 a | 12.32 b | 29.32 b | n.s | 11.16 |
| *Parkia speciosa* | 0.48 c | 0.57 b | 1.27 c | 2.33 d | n.s | 0.87 |
| *Acacia auriculiformis* | 2.55 b | n.s | n.s | n.s | 20.43 c | 3.98 |
| **DF11** | | | | | | |
| *Acacia crassicarpa* | n.s | n.s | n.s | 61.92 a | 82.00 a | 81.20 |
| *Falcataria moluccana* | n.s | 12.50 a | 27.47 a | 54.93 a | 73.00 a | 47.93 |
| *Archidendron pauciflorum* | 0.35 c | 0.35 b | 3.37 c | 4.42 c | 6.92 e | 2.19 |
| *Leucaena leucocephala* | 5.58 a | 11.17 a | 16.53 b | 36.63 b | 54.27 b | 19.69 |
| *Parkia speciosa* | 0.25 c | 0.48 b | 1.58 c | 11.97 d | 12.27 d | 5.79 |
| *Acacia auriculiformis* | 2.83 b | n.s | n.s | n.s | 21.28 c | 5.05 |

Note: n.s: No sample, cfu: colony-forming unit. [a] *F. oxysporum* population calculated at the end of the experiment (21 days after inoculation). [b] DI 0–4; 0: no disease/healthy seedling; 1: yellow leaves; 2: yellow leaves and slightly wilted; 3: severe wilt; and 4: dead seedling. [c] Average of *F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4: population of pathogen in score 0, 1, 2, 3, and 4: A: number of plants on score 0; B: number of plants on score 1; C: number of plants on score 2; D: number of plants on score 3; E: number of plants on score 4; N: total number of plants. [d] *F. oxysporum* isolates. [e] Values followed by the same letter in each row are not significant.

**Table 3.** *Fusarium oxysporum* population average and diseases index of plant

| Plant species | Population average (×10$^4$ CFU/g fresh weight) [a] | | | Disease index [b] | | |
|---|---|---|---|---|---|---|
| | AF01 [c] | BF05 | DF11 | AF01 | BF05 | DF11 |
| *Acacia crassicarpa* | 85.13 | 92.61 | 81.20 | 4.00 | 3.48 | 3.96 |
| *Falcataria moluccana* | 76.50 | 43.85 | 47.93 | 3.44 | 3.04 | 2.80 |
| *Archidendron pauciflorum* | 5.06 | 3.60 | 2.19 | 1.96 | 1.88 | 1.40 |
| *Leucaena leucocephala* | 22.13 | 11.16 | 19.69 | 1.52 | 1.56 | 1.68 |
| *Parkia speciosa* | 2.16 | 0.87 | 5.79 | 1.80 | 1.04 | 2.16 |
| *Acacia auriculiformis* | 4.65 | 3.98 | 5.05 | 0.36 | 0.40 | 0.60 |

Note: [a] Average of *F. oxysporum* population (cfu/g fresh weight): ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4: population of pathogen in score 0, 1, 2, 3, and 4: A: number of plants on score 0; B: number of plants on score 1; C: number of plants on score 2; D: number of plants on score 3; E: number of plants on score; N: total number of plants. [b] DI 0–4; 0: no disease/healthy seedling; 1: yellow leaves; 2: yellow leaves and slightly wilted; 3: severe wilt; and 4: dead seedling. [c] *F. oxysporum* isolates

**Discussion**

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Soleha et al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect Fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *Fusarium oxysporum* is able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the Fabaceae family had great potential to become an alternative host and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had become virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the proliferation and adaptation of the pathogens to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotype showed a population level of $15.4 \times 10^5$ CFU $g^{-1}$. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score. A similar result was also occurred in garlic with disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defense mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomenon was reported by Muslim et al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* were lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonization and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the induction of cell wall-associated phenolic compounds. Jiménez-Fernández et al. (2013) also reported that *Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proven to be an alternative host of *F. oxysporum*. Whereas *L. leucocephala*, *A. pauciflorum*, *P. speciosa*, and *A. auriculiformis* had potential as alternative hosts. Many plants of Fabaceae family were attacked by formae specialis *F. oxysporum*, such as *Vigna angularis* (*F. oxysporum* f. sp. *adzukicola*), *Cicer arietinum*, *Cicer* spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp. *koae*), *Lens culinaris*, *L. esculenta* (*F. oxysporum* f. sp. *lentis*), *Medicago sativa* (*F. oxysporum* f. sp. *medicaginis*), *Phaseolus vulgaris*, *P. coccineus* (*F. oxysporum* f. sp. *phaseoli*), *Pisum sativum*, *Cicer arietinum* (*F. oxysporum* f. sp. *pisi*) (Edel-Hermann and Lecomte 2019). However, in this study, *F. oxysporum* isolated from *A. mangium* has a wide host range from Fabaceae family; therefore, it is not classified as formae specialis.

In conclusion, *F. oxysporum* isolated from *A. mangium* causes infection in several types of forest and industrial plants. Since it has a wide host range, it is not classified as part of the formae specialis group.

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