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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.

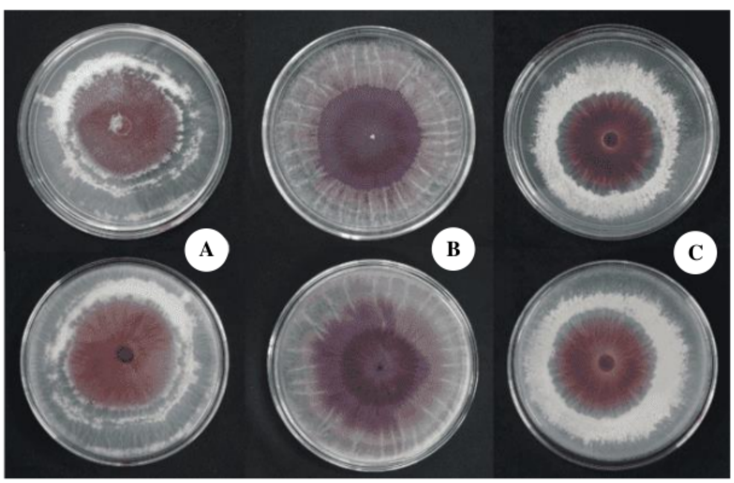

Figure 1. *Fusarium oxysporum* isolates on PDA medium. A. AF01, B. BF05, and C. DF11. First line: front view; second line: reverse view

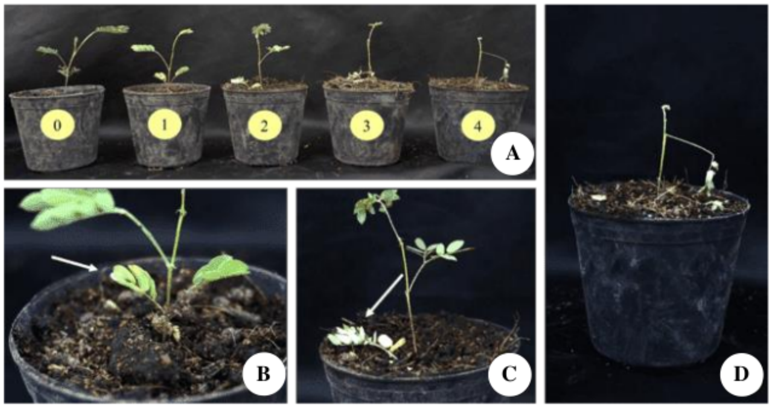

Figure 2. Disease index of *Acacia crassicarpa*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: falling leaves; D. Dead plant

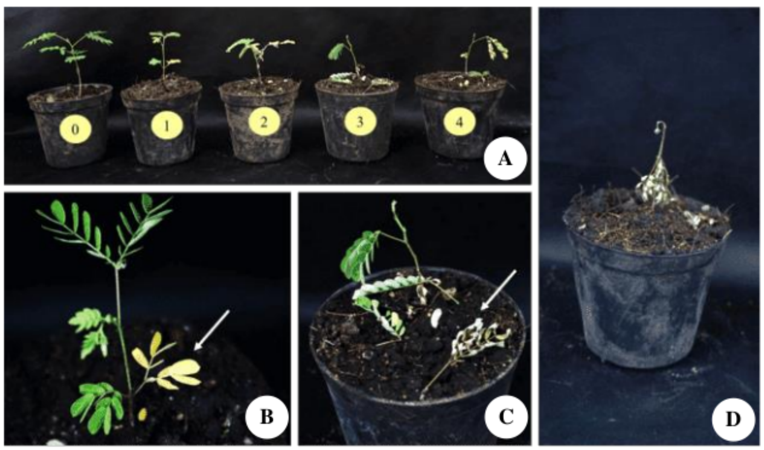

Figure 3. Disease index on *Falcataria moluccana*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: curved, dry, and falling leaves; D. Dead plant

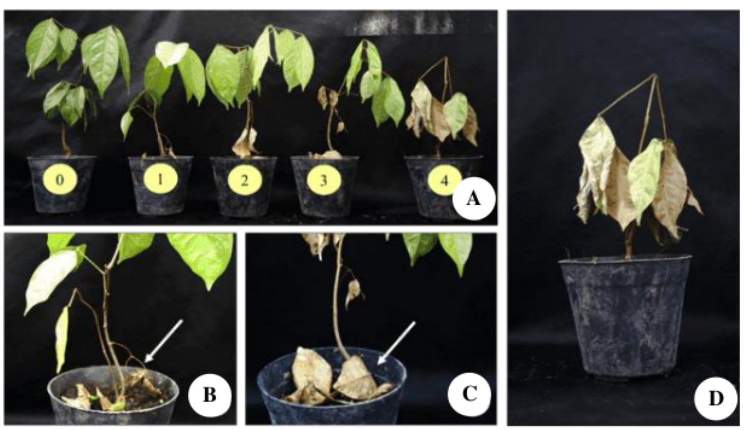

Figure 4. Disease index on *Archidendron pauciflorum*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing and dry from oldest leaves; C. Advanced symptoms: falling leaves; D. Dead plant

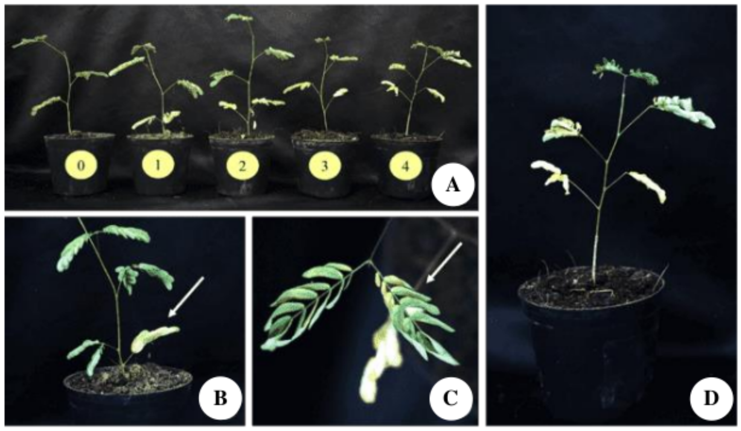

Figure 5. Disease index on *Leucaena leucocephala*, A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: curved leaves; D. Yellowing upward

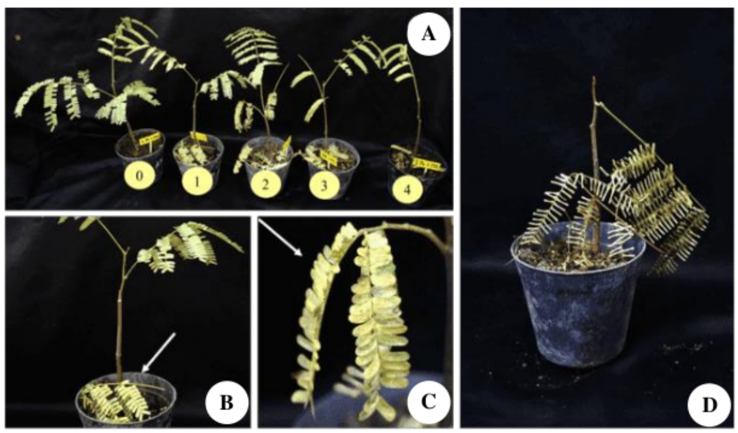

Figure 6. Disease index on *Parkia speciosa*. A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing and dry from oldest leaves; C. Advanced symptoms: curved leaves; yellowing, D. Dead plant

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*).

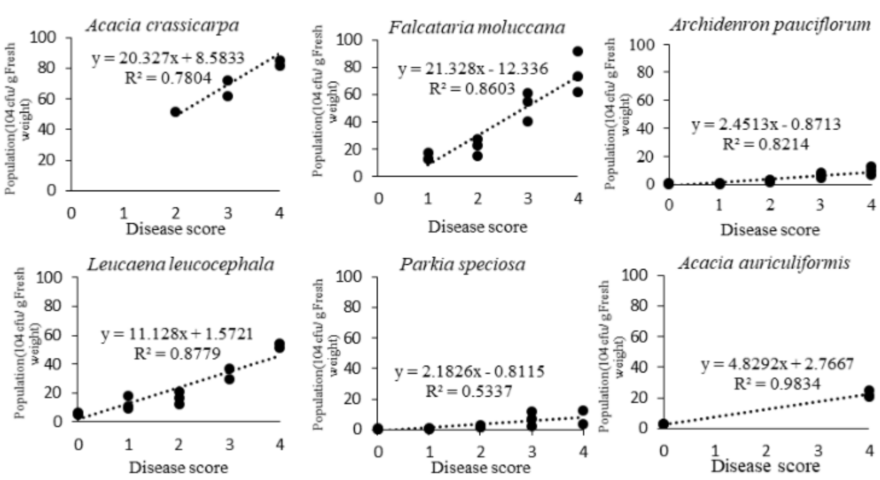

Figure 8. Regression analysis of disease score rate and *Fusarium oxysporum* population

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* ($\times 10^4$ 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 ($\times 10^4$ 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 × 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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