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Resistance mechanisms of white jabon seedlings (*Anthocephalus cadamba*) against *Botryodiplodia theobromae* causing dieback disease

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Abstract. *Yanti LA, Achmad, Khumaida N. 2018. Resistance mechanisms of white jabon seedlings (*Anthocephalus cadamba*) against* Botryodiplodia theobromae *causing dieback disease. Biodiversitas 19: 1441-1450. Anthocephalus cadamba* (Roxb.) Miq. seedlings are the most preferred plant for the nursery as they serve a lot of benefits and can be used as shading trees, reforestation, plywood, pulp, paper, and traditional medicines. Further, those benefits can increase the economic value of this plant. The main problem in the nursery of forestry plants is pest and disease attacks, one of which is dieback disease. The dieback disease is caused by *Botryodiplodia theobromae* Pat. that may lead death of the host plant. Every plant has its resistance mechanism toward pathogen attacks. This research aimed: (1) to study *B. theobromae* attack through wounded and non-wounded stem infection methods on white jabon seedlings; (2) to study the resistance mechanisms of white jabon seedlings both structural and biochemical resistance against *B. theobromae*. This study employed a factorial treatment design laid out in a completely randomized design. The structural resistance was determined by studying the microscopic appearance of the white jabon seedlings' stem by using a scanning electron microscope. Meanwhile, the biochemical resistance was determined by characterizing the chemical compounds of white jabon seedlings' stem using phytochemistry analysis. The result showed that the disease incidence of the control (inoculated without pathogen isolate) and the inoculated (inoculated with pathogen isolate) seedlings were, respectively, 0% and 100% (with wounded stem) and 0% and 30% (non-wounded). The disease severity of control and inoculated seedlings were 0% and 62% (with wounded stem) and 0% and 12% (non-wounded stem), respectively. The incubation period of wounded and non-wounded stems on inoculated seedlings (inoculated with pathogen isolate) was one day after inoculation with the numeric values (disease scores) of 4 and 2, respectively. White jabon seedlings had necrotic resistance as structural resistance mechanism against the pathogen attack. White jabon seedlings also contained secondary metabolites such as alkaloids, flavonoid, phenyl hydroquinone, tannin, saponin, and steroids. The biochemical resistance of white jabon seedling after pathogen attacks was shown by the increase of accumulated phenolic compounds such as flavonoid and tannin.

Keywords: *Anthocephalus cadamba*, histopathology, necrotic resistance, phenolic compounds, stem infection

INTRODUCTION

White jabon (*Anthocephalus cadamba* (Roxb.) Miq.) is a fast-growing plant, which is the most preferred forestry plant nowadays. This jabon is mostly used as shading trees, an ornament for the curb, and in reforestation (Orwa et al. 2009). It can also be used for plywood, light construction, floor, pulp, paper, ceiling, box, toy, engraving, and traditional medicine. The white jabon timber is categorized into the strength classes of III-IV and durability class of V. According to Oey (1990), the durability classes of timber are grouped into 5 categories, i.e., highly resistant (I), resistant (II), moderate (III), not durable (IV), and highly not durable (V). Because of its benefits and excellence, white jabon is widely cultivated at the level of nursery (Sudrajat 2015).

The main problem that often occurs in forestry nursery is pest and disease attacks. The diseases that most frequently occur in the forestry nursery are dieback, leaf spot, and leaf blight. This research focused on dieback disease caused by *Botryodiplodia* sp. According to Kunz (2007), *Botryodiplodia* sp. is included in Deuteromycetes and a saprophyte. Molecular identification by Winara (2014) showed that the pathogenic species causing dieback disease is *Botryodiplodia theobromae*. According to Anggraeni and Lelana (2011), *Botryodiplodia* sp. was reported as pathogen of some forestry plants in Indonesia, causing leaf spots on *Alstonia* sp., *Intsia bijuga* Kuntze., *Rhizophora mucronata* Lamk., *Macaranga gigantea* Muell., root rot on *Shorea* sp., *blendok* on *Calophyllum inophyllum* Linn., and stem disease on *Aquilaria malaccensis* Lamk. Begoude et al. (2009) also stated that *Botryosphaeriaceae* has a very wide host distribution on *monocotyledon*, *dicotyledon*, *Gymnospermae*, and *Angiospermae*. This pathogen can only infect the host plants through wounds or injuries, but severe infections may occur (Semangun 2007). According to Aisah (2014), *B. theobromae* could attack white jabon seedlings through wounded and non-wounded stem infection methods.

Botryodiplodia spp can infect four months old the white jabon seedlings through non-wounded stem infection method (Aisah 2014). Besides, Arshinta (2013) showed that white jabon seedlings aged 3, 4, and five months suffered disease incidence of 100% with disease severity of

61.42, and 54%, respectively. Dieback disease that occurs on white jabon seedling can cause destructions and the death of the seedling, which may lead to the reduction of economic benefits. Healthy trees are derived from quality seedlings which are not infected by pests and diseases. According to Achmad et al. (2012), the increase of pine seedling age could cause the rise in resistance to seedling rot disease. This research, therefore, employed five months white jabon seedlings for evaluation of their resistance mechanisms against *B. theobromae*.

Studies about *Botryodiplodia* spp. that attacks white jabon seedlings had been previously carried out by many workers. These workers included Arshinta (2013) on the pathogenicity test of *Botryodiplodia* sp. on white jabon seedling, Aisah (2014) on the virulence test of *Botryodiplodia* sp. on white jabon seedling (*A. cadamba*) and Winara (2014) on the bioactivity test of mahogany extract and molecular identification of *Botryodiplodia* sp. on white jabon seedlings. However, studies on resistance mechanism of white jabon seedling against *Botryodiplodia* sp. have never been done.

Every plant has its own resistance mechanism against pathogens. According to Agrios (1997), the disease resistance mechanisms can be distinguished into two categories, namely structural and biochemical resistance, which occur both before and after the pathogen attack. Structural resistance includes the surface structures of plants and tissues, cells, cytoplasm, and necrotic resistance mechanism. Biochemical resistance includes the existence of inhibitor substances in plant cells such as secondary metabolites and the increase in accumulation of phenolic compounds. Some secondary metabolites are the phenolic compound such as flavanoid, quinone, and tannin. Research carried out by Wali (2014) showed that the leaves of white jabon seedlings contained secondary metabolites, namely quinone, and steroid.

Based on the above description, research on the resistant mechanism of white jabon seedling against *B. theobromae* attack is essential. Therefore, this study aimed to: (i) study *B. theobromae* attack white jabon seedlings through wounded and non-wounded stem infection methods; (ii) investigate the resistance mechanism of white jabon seedlings, both structurally and biochemically, against *B. theobromae* attack.

MATERIALS AND METHODS

This research was conducted from April to December 2014 in the Laboratory of Forest Pathology, Faculty of Forestry, and Laboratory of Analytical Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB), Bogor, Indonesia, plants nursery of BPDAS Citarum-Ciliwung, Dramaga, Bogor, Indonesia and Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia.

Procedures

Rejuvenation and multiplication of isolated B. theobromae

The isolates of *B. theobromae* is a collection of Forest Pathology Laboratory, Bogor Agricultural University, Bogor, Indonesia (Aisah 2014). Isolate multiplication was done by purifying the existing isolates. Rejuvenation was done based on Michailides's (1991) modification. The pathogen was planted on a PDA using a 5 mm diameter corebore, and then was incubated at 25°C in Laminar Air Flow until the pathogen growth filled the petridish. The pure culture of *B. theobromae* isolates was then used as the inoculum source.

Macroscopic and microscopic observations of B. theobromae isolate

The observation was done based on the morphological characteristics of macroscopic and microscopic elements such as color, texture, colony topography, growth diameter, size, and hyphae shape. Identification was carried out following the book of fungi identification key for imperfect fungi (Barnet and Hunter 1998).

Research design

This research used a factorial treatment design assigned in a complete randomized design that combined pathogen inoculations (control and inoculated with pathogen isolate) and stems infection methods (wounded and non-wounded) in seedlings with 10 replications. Samples were placed in paranets and arranged accordingly to suit the treatment design.

Resistance evaluation

White jabon seedlings aged five months were used in this research. Seedlings were obtained from a nursery in Bogor with the origin of provenance in Malang. Seedlings were selected to have the same height, diameter, and the number of leaves, healthy, and all in good condition. Evaluation of resistance was carried out using a jelly block pasting method based on Ismail et al. (2012) with modification. In the wounded stem infection method, the inoculation was carried out using syringes. The control treatment was inoculated with jelly block without the fungal pathogen isolate. A seven-day-old fungal isolate was used in the inoculation treatment. The observations were done for 14 days. In the non-wounded infection method, inoculation was done on the side of the stem with no any lenticels (examined using a loop). The observed parameters were the disease incidence (Achmad et al. 2012) and disease severity (Townsend and Heurberger (1943) *in* Stevic et al. (2010), the incubation period, temperature and air humidity in the nursery (in the morning, at noon and night).

Structural resistance analysis of white jabon seedling by using Scanning Electron Microscopy (SEM)

Stems of healthy white jabon seedling were used as samples of control treatment and infected stems of white jabon seedlings through wounded and non-wounded stem infection methods. The analysis was done based on the Guide Book of Zoology Research Center, Indonesia

Science Institution, Cibinong. The samples were observed using a scanning electron microscopy (model JSM-5310LV).

Biochemical resistance of secondary metabolite analysis of white jabon seedling by using phytochemical analysis

The analysis was done following the method of Harborne (1998) on the stem of healthy white jabon seedlings as control treatment and infected white jabon seedlings through wounded stem infection methods. Samples from white jabon seedling's stem were used in a powder of 500 mg in each testing. The secondary metabolite analysis included alkaloids, flavonoid, phenyl hydroquinone, tannin, saponin, triterpenoid, and steroid assays.

Alkaloids assay. The sample was dissolved in 5 mL chloroform and added with five drops of NH4OH, and the solution was then shaken and filtered. Two mL of chloroform extract was added with 10 drops of 2 M H_2SO_4, and then stirred until two layers were formed. The top layer (the acid) was taken and dropped on the plate and assayed with Meyer reagent, Wagner reagent, and Dragendorff reagent. The assay would be positive if a white, brown and red-orange color of precipitates appear.

Flavanoid assay. The sample was added with 10 mL of hot water, then boiled for 5 minutes and filtered. Five mL filtrate was added with 0.5 g Mg, 1 mL HCl, and 1 mL of amyl alcohol, and then shaken. The assay would be positive if the solution shows yellow until deep red colors.

Phenol hydroquinone assay. The sample was added with 10 mL methanol, and then shaken and boiled in hot water and filtered. The filtrate was added with three drops of 10% NaOH. The test would be positive if the solution showed yellow until red colors.

Tannin assay. The sample was added with 50 mL of hot water and boiled for 15 minutes and then filtered. The filtrate was added with 10 mL of 1% $FeCl_3$. The assay would be positive if green until black colors appear.

Saponin assay. The sample was boiled in 10 mL of hot water for 5 minutes and filtered. 10 mL of filtrate was shaken in a reaction tube for 10 seconds and then left at room temperature for 10 minutes. The assay would be positive if stable foam is produced.

Steroids and triterpenoids assay. The sample was macerated in 25 mL of absolute ethanol until boiled up, and then filtered. The residue was boiled to dry it up and then was added with ether and shaken. The filtrate was added with three drops of anhydrous acetic acids and one drop of sulphuric acid. The solution was whipped and let off for a few minutes. The appearance of red and purple colors demonstrates a positive assay for triterpenoids, and green or blue shows a positive test for steroids.

Data analysis

Disease incidence and severity data were subjected to analysis of variance, which then followed by the Tukey test to separate the treatment means when the treatment effect was significant. The analysis was performed by using Minitab 15. Structural and biochemical resistances data were descriptively analyzed and presented in forms tables and figures.

RESULTS AND DISCUSSION

Macroscopic and microscopic characteristics of *B. theobromae* isolates

This pathogen had white colonies on the culture surface (Figure 1.A), which later turned grey or blackish green (Figure 1.B). The color of the bottom of the media was grey, blackish green or black (Figure 1.C). Mycelium of *B. theobromae* had a fluffy texture with thick air mycelium, and the colony spread from the central part with irregular topography. Diameter growth rate of *B. theobromae* was fast with a mean of 1.38 mm hours $^{-1}$.

Botryodiplodia theobromae had septate and branched hyphae, hyaline when it was young, and brown when it was old; the size was 53-57 x 3-2 µm (Figure 1.D). Aisah (2014) showed that *B. theobromae* had hyaline conidia and nonseptate, which later became brown and septate when old. Conidia shape was ellipsoid or ovoid in size of 26-32 x 13-17 µm (Figure 1e). The macroscopic and microscopic characteristics of *B. theobromae* are shown in Figure 1.

Disease incidence and severity on white jabon seedling

The disease incidence on white jabon seedling in control treatment with wounded and non-wounded stem infection methods were all 0% (Figure 2.A and 2.E). The disease severity of white jabon seedling in control treatment with wounded and non-wounded stem infection methods were also 0%. Control treatment (inoculated without pathogen isolate) of white jabon seedlings showed no dieback symptoms.

The disease incidence on white jabon seedling in inoculated with pathogen isolate treatment through wounded (Figure 2.B, 2.C, and 2.D) and non-wounded (Figure 2.F, 2.G, and 2.H) stem infection methods were 100% and 30%, respectively. The disease severity of white jabon seedling in plants inoculated with pathogen isolate treatment through wounded and non-wounded stem infection methods were, respectively, 62% and 12%. The dieback disease symptoms observed on white jabon stems were decaying marks on the stem, wilting on the leaf, drying up of stem and leaf, necrosis and dieback. The conditions of white jabon seedling with wounded and non-wounded stem infection methods are presented in Figure 2.

The response of pathogen inoculations and stem infection methods in white jabon seedlings showed that the disease incidence on the plants inoculated with pathogen treatment through wounded stem infection was significantly different from those inoculated with the pathogen through non-wounded stem infection and the control treatment (both wounded and non-wounded stem infection methods) (Table 1). The disease incidence on white jabon seedlings inoculated with the pathogen through wounded stem infection (100%) was more extensive than that on white jabon seedlings inoculated with the pathogen through non-wounded stem infection (30%).

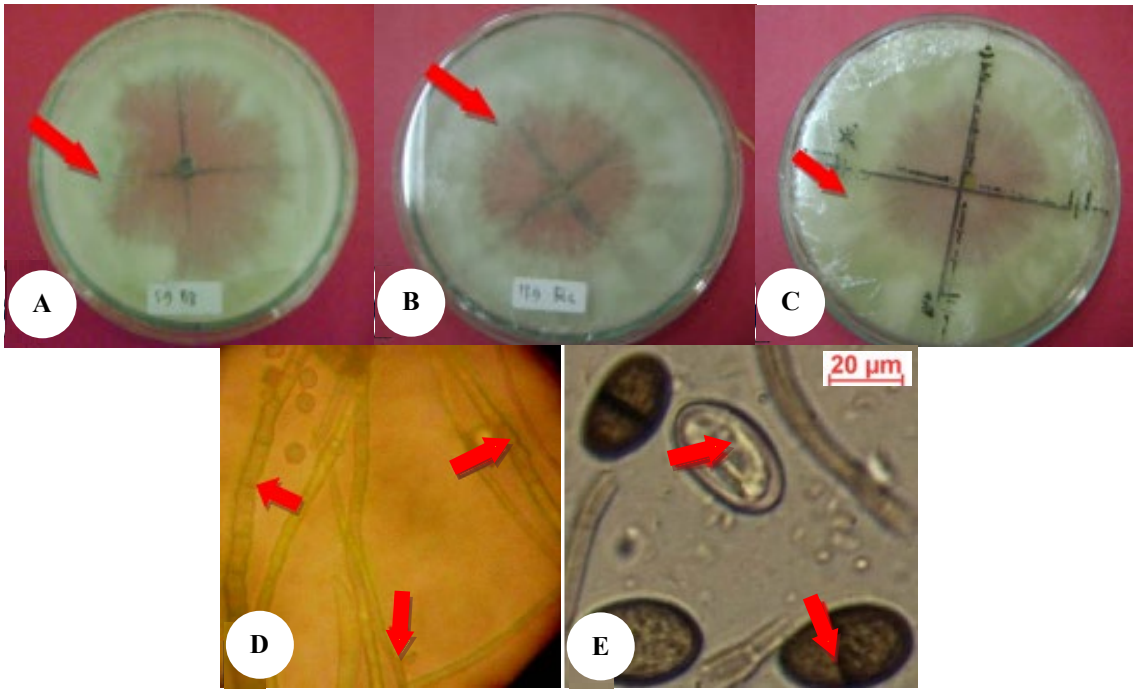

Figure 1. Macroscopic and microscopic characteristics of *B. theobromae*. A. White colonies on culture surface; B. Grey colonies; C. The bottom of media looked grey; D. Septate hyphae; E. Conidia shape (Aisah 2014)

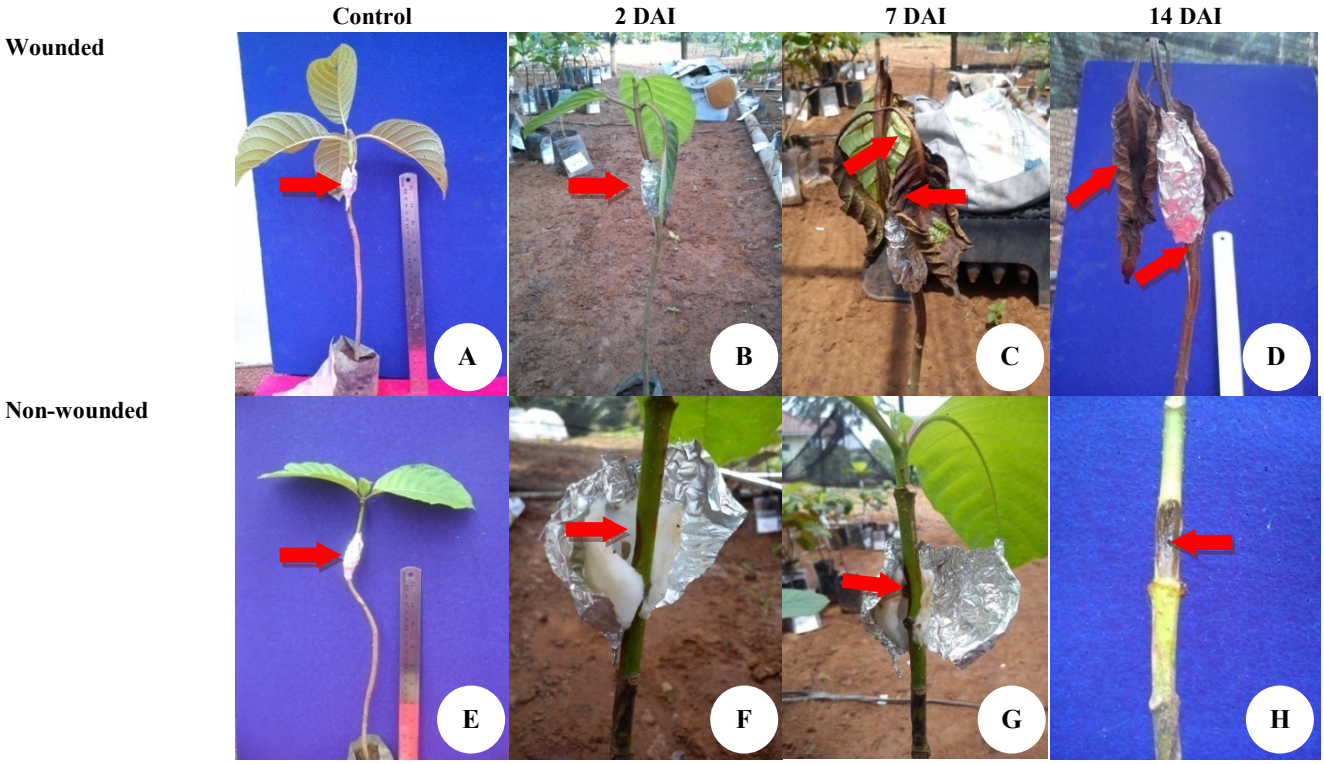

Figure 2. The condition of white jabon seedling during 14 days of observation. A. Control treatment with wounded stem infection method; B. 2nd day after inoculation (DAI) through wounded stem infection method; C. 7th DAI after inoculation through wounded stem infection method; D. 14th DAI after inoculation through wounded stem infection method; E. Control treatment with non-wounded stem infection method; F. 2nd day after inoculation (DAI) through non-wounded stem infection method; G. 7th DAI after inoculation through non-wounded stem infection method; H. 14th DAI after inoculation through non-wounded stem infection method

The disease severity on white jabon seedlings inoculated with the pathogen through wounded stem infection was significantly different from those inoculated with the pathogen through non-wounded stem infection method and the control treatment (both wounded and non-wounded stem infection) (Table 1). The disease severity on white jabon seedlings inoculated with the pathogen through wounded stem infection (62%) was worse than that on white jabon seedlings inoculated with the pathogen through non-wounded stem infection (12%). The response of pathogen inoculations and stem infection methods on white jabon seedlings are presented in Table 1.

Incubation period

The incubation period is time intervals from inoculation to the appearance of disease symptoms. The incubation period of white jabon seedling was one day after the inoculation. The number of infected white jabon seedlings during 14 observation days was none for control, ten for inoculation with the pathogen treatment through wounded stem infection and three for inoculation with the pathogen treatment through non-wounded stem infection. On the 14th days after pathogen inoculation, the numeric value of disease category and disease severity of white jabon seedlings, both wounded and non-wounded stem infection were, respectively, 4 and 2. The incubation periods are presented in Table 2.

Temperature and air humidity

Temperature and air humidity are the most important environmental factors for the growth of both the pathogens and the host plant, that will affect the development of the disease. The mean temperature for 14 days of observation was 27.53 °C in the morning, 33.27 °C during daytime and 26.07 °C at night. Table 3 shows the temperature and air humidity during 14 days of observation.

Table 3. Mean temperature and air humidity during 14 days of observation

Day after inoculation	Temperature (°C)			Air humidity (%)		
	Morning	Day	Night	Morning	Day	Night
Inoculation day	29	33	25	78	73	92
1	28	29	29	85	78	85
2	29	35	26	78	69	92
3	29	36	25	78	75	92
4	27	32	23	92	73	91
5	29	34	25	78	74	92
6	27	35	25	92	69	92
7	26	33	27	84	73	100
8	30	33	26	85	67	100
9	27	36	25	92	75	92
10	23	31	29	83	86	85
11	29	36	27	85	56	92
12	26	32	27	92	73	92
13	27	31	26	84	73	100
14	27	33	26	84	73	92
Mean	27.53	33.27	26.07	84.67	72.47	92.60

Table 1. Mean dieback disease incidence and severity on white jabon seedling

Treatment	Replication	Disease incidence (%)*	Disease severity (%)*
White jabon (control/inoculated without pathogen), through wounded stem infection method	10	0^{b}	0^{b}
White jabon (control/inoculated without pathogen), through non-wounded stem infection method)	10	0^{b}	0^{b}
White jabon (inoculated with the pathogen) through wounded stem infection method	10	100^{a}	62^{a}
White jabon (inoculated with the pathogen) through non-wounded stem infection method	10	30^{b}	12^{b}

Note: *Means within the same column with the same letter are not significantly different at Tukey test (α = 95%)

Table 2. The incubation period of white jabon seedling against *B. theobromae* attack

Treatment	Number of infected seedlings	Incubation period (day)	The highest numeric value*
White jabon (control / inoculated without pathogen), through wounded stem infection method	0	0	0
White jabon (control / inoculated without pathogen), through non-wounded stem infection method)	0	0	0
White jabon (inoculated with the pathogen), through wounded stem infection method	10	1	4
White jabon (inoculated with the pathogen), through non-wounded stem infection method	3	1	2

Note: * = the highest numeric value of 10 replications recorded on the 14th days after inoculation. This value refers to the numeric value of disease category and dieback disease severity of white jabon seedlings (Townsend and Heurberger (1943) *in* Stevic et al. (2010)

Structural resistance in the white jabon seedlings' stem

White jabon seedlings demonstrated necrotic resistance by activating hypersensitivity reaction, which is a form of structural resistance in response to the pathogen attack. The healthy white jabon seedlings (control) showed no hyphae of *B. theobromae* fungi (Figures 3.A and 3.B). White jabon inoculated with pathogen treatment (infected) through wounded stem infection method showed the destruction of epidermal tissues, cortex, and stele up to epidermal tissues and stele on the opposite side (Figure 3c). White jabon seedlings inoculated with the pathogen (infected) through non-wounded stem infection method showed the destruction of epidermal tissues, cortex and hyphae colonization on the stele (Figures 3e and f).

Figure 3 shows the microscopic of the transverse section of white jabon seedling's stem using scanning electron microscope.

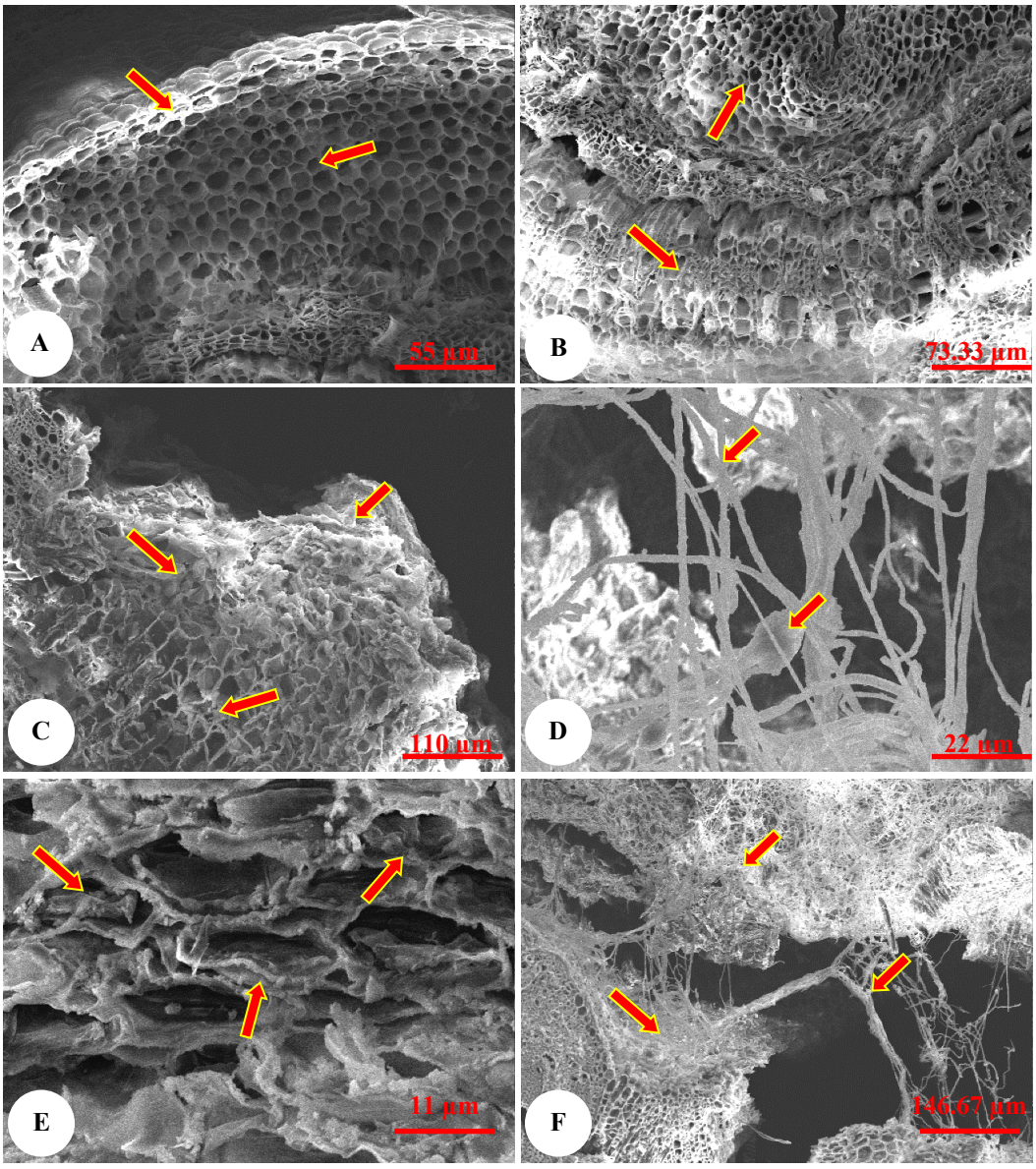

Figure 3. Electronic micrographs of white jabon seedling's stem. A. epidermal and cortex tissues of healthy white jabon seedling; B. stele tissues of healthy white jabon seedling; C. epidermal, cortex, and stele tissues, which were dried and detached from the host (white jabon seedling's stem inoculated with pathogen through wounded stem infection method), and the pathogen infected part up to the cortex and epidermal tissues in the opposite side; D) hyphae of *B. theobromae*; E) cortex of destructed white jabon seedling inoculated with pathogen through non-wounded stem infection method; F) juvenile mycelium of *B. theobromae* attacking the stele tissues of white jabon seedling inoculated with the pathogen through non-wounded stem infection method

Table 4. Secondary metabolites of white jabon seedlings' stem

Active compounds	Treatment	
	Control (healthy, inoculated without pathogen)	Inoculated with pathogen (infected)
Alkaloids	++	+
Flavonoid	++	+++
Phenol hydroquinone	+++	++
Tannin	+++	++++
Saponin	+++	++++
Triterpenoid	-	+
Steroid	+	+++

Note: (-) negative, (+) positive but weak, (++) positive and somewhat strong, (+++) strong positive, (++++) very strong positive.

The biochemical resistance of white jabon seedlings' stem

A healthy white jabon seedling contained secondary metabolites such as alkaloids, flavonoid, phenyl hydroquinone, tannin, saponin, and steroid but contained no triterpenoid. In the seedlings inoculated with the pathogen (infected), there was an increase in some phenolic compounds such as flavonoid and tannin. This treatment also demonstrated an increase in saponin, triterpenoid and steroid contents. The secondary metabolites of white jabon seedling are presented in Table 4.

Discussion

Isolates of *B. theobromae* had a fast radial growth, that was able to fulfill the petridish (d= 9 cm) in day three - day four after incubation. Diameter growth rate was rapid, with a mean of 1.38 mm hours^{-1}. According to Winara (2014), mycelium growth of *Botryodiplodia* sp. was fast, with mean radial diameter growth of 1.72 mm hours^{-1}.

During one month incubation period, we found no any pycnidium of *B. theobromae*. According to Kunz (2007), the pycnidia development of *Botryodiplodia* spp. in artificial media takes a long time, but when it grows, the large black and round pinhead can be seen directly. Research results of Shah et al. (2010) showed that 13 isolates of *B. theobromae* grown in PDA media could form pycnidia on the 20-34th incubation days.

The mycelium of *B. theobromae* had septate and branched hyphae, and the hyphae were hyaline at the juvenile stage and brown and the mature/old stage with a size of 53-57 x 3-2 µm. Aisah (2014) showed that *B. theobromae* had hyaline conidia and nonseptate, which later became browning and was septate when getting older. The shape of the conidia was ellipsoid or ovoid with a size of 26-32 x 13-17 µm. Kumar and Leena (2009) stated that *B. theobromae* had a hyaline mycelium, septate, branched, and sized 50-55 x 3-4 µm.

The disease incidence and severity of white jabon seedlings in the control treatment were 0%. White jabon seedling inoculated without pathogen (control) showed no dieback symptoms. According to Arshinta (2013), white jabon seedling aged 3, 4, and five months, showed symptoms of dieback disease; nevertheless, white jabon seedling inoculated without pathogen (control) showed no dieback disease symptoms.

The disease incidence of white jabon seedling inoculated with the pathogen through wounded and non-wounded stem infection were, respectively, 100% and 30%. The disease severity of white jabon seedling inoculated with the pathogen through wounded and non-wounded stem infection was 62% and 12%, respectively. The disease incidence on the seedlings inoculated with pathogen treatment through wounded stem infection method was wider than that on the seedlings inoculated with the pathogen through non-wounded stem infection method. The disease severity on the seedlings inoculated with pathogen through wounded stem infection method was worse than the those inoculated with pathogen through non-wounded stem infection. According to Semangun (2007), *B. theobromae* is a weak pathogen that needs injuries/wounds to infect the host, but it can be a serious disease. Although the disease incidence and severity on the seedlings inoculated with the pathogen through non-wounded stem infection method had a higher value (30% and 12%) than control (0% and 0%), they were not significantly different.

In this research, we employed five months old white jabon seedlings. Age of the plant could increase the resistance mechanism of the plant. According to Achmad et al. (2012), the increase of pine seedling age could cause the increase of resistance of seedling to root rot disease. Arshinta (2013) showed that white jabon seedlings aged 3, 4, and five months had disease incidence of 100%, and the disease severity was, respectively, 61%, 42%, and 54%.

The incubation periods of white jabon seedlings inoculated with the pathogen through both wounded and non-wounded stem infection methods were similar, i.e., one day after inoculation. The number of infected white jabon seedling during 14 observation days were none for the control treatment, ten for seedlings inoculated with pathogen treatment through wounded stem infection method and three for those inoculated with pathogen treatment through non-wounded stem infection method. Research by Sato et al. (2008) employing *Corchorus olitorius* seedlings inoculated with seven days old inoculum of *L. theobromae* showed the disease symptoms on 7-10th days after inoculation.

Environmental conditions important factors for the development of a disease are the temperature and air humidity. Means of temperature recorded during 14 days of observation were 27.53°C in the morning, 33.27°C in the daytime and 26.07°C at night. Means of air humidity recorded during 14 days observation were 84.67% in the morning, 72.47% at daytime, and 92.60% at night. During 14 days of observation, white jabon seedlings were in the optimum temperature, so the symptom occurred was caused by the biotic factors. According to Martawijaya et al. (1989), the maximum temperature for jabons' growth was 32-42°C and the minimum temperature was 3-15.5°C. These temperature and humidity levels were also the optimum temperatures for the *B. theobromae* fungal

pathogen. According to Sato et al. (2008), *L. Theobromae* optimally grows at a temperature of 30°C.

White jabon seedlings had necrotic resistance through hypersensitivity reaction, which is a structural resistance mechanism that is activated after the pathogen attack. White jabon inoculated with pathogen treatment (infected) through wounded stem infection method on 14th DAI, *B. theobromae* attacked epidermal tissues, cortex, and stele until epidermal tissues and stele on the opposite side. The damaged tissues showed necrosis, dried, and detached from the host. In the white jabon seedlings inoculated with the pathogen (infected) through non-wounded stem infection method, on the 14th DAI, *B. theobromae* attacked the epidermal tissues, cortex and still on the stele. According to Agrios (1997), one of the active plant structural resistances is necrotic resistance activated through hypersensitivity reaction. The hypersensitive response causes damage to cellular membrane infected by the pathogen. It causes the plant tissues to respond to the pathogen by producing necrosis symptom. Saadon et al. (2012) found that, on seven days after inoculation, grapes epidermal cells were attacked by *L. theobromae*. The cortex and xylem tissues were colonized by hyphae of the fungal pathogen. In 25 days after inoculation, the pathogen spread out inter-cell of all tissues. Plasmolysis was observed to occur in epidermal cells and cortex, and gummosis was formed on xylem. In 30 days after inoculation, grape seedlings became dark brown in color, pulpy, withered, and died.

Infection of *B. theobromae* on white jabon seedlings occurred through the lenticel, artificial wounds, and also direct infection through the epidermal surface using physical or biochemical weapons. According to Aisah (2014), the infection mechanism of *Botryodiplodia* spp. on white jabon seedlings occurred through the production of pectinase and cellulase enzymes that degrade the cell walls. After the mycelium came into the cell, it destroyed epidermal, the cortex, and the stele tissues until the epidermal and cortex tissues are broken through the opposite side.

The biochemical resistance of white jabon seedlings did occur through the existence of inhibitory substances in the host cells such as secondary metabolite compounds. White jabon seedlings contained alkaloids, flavonoid, phenyl hydroquinone, tannin, saponin, and steroid compounds. According to Verpoorte and Alfermann (2000), secondary metabolite is a compound of non-essential metabolites that serve as a resistance mechanism to environmental conditions, resistance to pest attacks, diseases, and attracts the pollinators.

The stem of white jabon seedling of five months old in the control treatment contained alkaloids, flavonoid, phenyl hydroquinone, tannin, saponin, and steroid. Wali (2014) found that leaves of white jabon aged seven months only contained quinone and steroid compounds. The contents of saponin and phenolic such as the flavonoid, phenyl hydroquinone, and tannin can be different depending on the types, age, and parts of the plants. According to Haralampidis et al. (2002), the contents of saponin in plants depend on several factors such as genetic of the plants, kind of tissues, age and the physiological state of the plants, and the environment.

Biochemical resistance after pathogen attack of white jabon seedling occurred through the increase in phenolic compounds accumulation. Several compounds of secondary metabolites included the phenolic compound such as the flavonoid, tannin, and phenol hydroquinone. The inoculated white jabon seedlings showed an increase of flavonoid and tannin compounds. According to Agrios (1997), one of the plant biochemical resistances after pathogen attack is the increase in the accumulation of phenolic compound. The rise of accumulation of phenolic compound occurs soon after pathogen infection in resistant varieties. Widnyana et al. (2009) found an increase of total phenol content in tomatoes infected by *Fusarium* sp. According to Shaul et al. (2001), the rise in flavonoid plays roles in synthesizing chitinase enzymes and phenylalanine ammonium lyase. Mechanism of tannin as an antibacterial substance is by shrinking the membrane cell, and then interfering the cell permeability that may lead to cell death, protein precipitation, inactivation of enzyme, and destruction of the function of genetic material (Ajizah 2004).

Besides, we found in the present study that white jabon seedlings also showed an increase of saponin, triterpenoid and steroid compounds. According to Astawan and Kasih (2008), saponin serves as an antimicrobial substance, alcoholic drink, textile, cosmetics, and traditional medicines. Winara (2014) demonstrated that extract of leaves, bark, rind of the fruit, seeds, and roots of mahogany had antifungal compounds against *Botryodiplodia* sp. in in-vitro assay because these extracts contain limonoid derived from limonin and triterpenoid. Triterpenoid of annual plant roots, stems of two years old, leaves, flowers, and twigs of *Jatropha curcas* are potential antifungal compound against *M. albican* and *C. guiliermondii* (Lei et al. 2015). According to Bayu (2009), steroid serves as anti-inflammation, anticarcinogenic, and controller of diabetes. Kristanti et al. (2008) also added that steroid could be used as a toxic compound.

Research by Hardiningtyas (2009) showed that mechanism of saponin as an antifungal compound does occur through its interaction with sterol. Wink (2013) showed that the mechanisms of triterpenoid as antifungal compound occurs through its interaction with biomembranes, which may cause the leakage of the fungal cells' ions. The antifungal mechanism of steroid does occur through its interactions with biomembranes.

White jabon seedling also had a decrease in alkaloids and phenol hydroquinone. This would have happened because the function of secondary metabolites is not only as antimicrobial agent. Alkaloids serve as an α-glucosidase enzyme inhibitor (Samson 2010), in the health sectors (Aksara et al. 2013), and as an antioxidant (Yuhernita and Juniarti 2011). Rastuti and Purwati (2012) showed that phenol hydroquinone has an antioxidant activity. Anthraquinone consists of *Morinda citrifolia* as an inhibitor of bacteria growth. According to Peoloengan et al. (2006), phenol is an antimicrobial compound.

According to Lamothe (2009), the mechanism of alkaloids as antibacterial is by disturbing peptidoglycan of bacterial cells, so that the cell walls are not fully formed and causes the death of cells. Mechanism of phenol as an antimicrobial is by destructing the cell walls of fungi, lysing, inhibiting the process of formation of the cell wall, changing the permeability of cytoplasm membrane, denaturating cell proteins, and destructing metabolism system by inhibiting the work of intracellular enzyme (Dwidjoseputro 1994).

The disease incidence of white jabon seedlings inoculated with the pathogen through wounded stem infection method was more extensive that of white jabon seedlings inoculated with the pathogen through non-wounded stem infection method. Similarly, the disease severity of white jabon seedlings inoculated with the pathogen through wounded stem infection method was also worse than that of white jabon seedlings inoculated with the pathogen through non-wounded stem infection method. White jabon seedlings do not have any structural resistance before pathogen attack, but it has necrotic resistance through hypersensitivity reaction as the resistance after pathogen attack. Biochemical resistances of white jabon seedling both before and after pathogen attack were found as secondary metabolites such as alkaloids, flavonoids, phenol hydroquinone, tannin, saponin and steroids, and the increase of phenolic compounds, such as flavonoids and tannin, and the increase of saponin, triterpenoids and steroids compounds.

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