Pathogenicity of *Botryodiplodia theobromae* Pat. on *Maesopsis eminii* Engl. Seedling Leaves

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Abstract. *Maesopsis eminii* Engl. is a commercial wood which classified as fast-growing multifunctional species. The disease will inhibit plant growth as well as decrease the quality of timber. *Botryodiplodia theobromae* disease-producing pathogen which is harmful and has many hosts. This study aims to examine the pathogenicity of *Botryodiplodia theobromae*, which harms the plant seedling leaves both macroscopically and microscopically. The experimental used a completely randomized design. The treatments consisted of inoculation without wounding, wounding, wounding with inoculation. The results showed that there was a symptom of brownish-colored spot occurred on plant seedling leaves. According to the Duncan’s multiple range test (DMRT), wounding with inoculation provided the greatest average value of disease severity by 27.56% and disease incidence by 82.67%. The results of macroscopic observation on PDA showed that colony of isolates until seven days old had white-colored mycelium, while the old mycelium looked grayish to black. Mycelium colony had a texture which is similar to the fine threads with thick aerial mycelium and dispersed unevenly from the middle part of mycelium. Microscopic observation on the symptomatic leaf disease pathogens result showed *Botryodiplodia theobromae* characteristics, such as sectional hyphae and ellipsoidal conidia hyaline.

1. Introduction

*Maesopsis eminii* Engl is an angiosperm that belongs to the Rhamnaceae family, which includes many extremely drought-tolerant species. *M. eminii* is considered to be a complex of four subspecies: *berchemioides, eminii, stuhlmannii* and *tessmannii* native to tropical Africa. It was reported as a human-introduced species outside of Africa, namely in Australia, Philippines, Bangladesh, Brazil, Costa Rica, Fiji, India, Malaysia, Samoa, Solomon Islands, Hawaii, Puerto Rico, and Indonesia [1, 2]. The sapwood is light-colored, heartwood brownish-olive to dark red, soft and light with a coarse grain. Wood density varies from 0.38 to 0.48 g/cubic cm. The wood dries rapidly, but logs have a tendency to split during felling and storage. The wood saws and machines easily, and its high absorbency makes it easy to treat with preservatives but difficult to finish. Therefore, this species is one type of commercial timber that is needed by various wood industries. The plants are classified as fast-growing species. They are used for light construction, boxes, containers, and plywood [3]. This species can also be combined in the agroforestry systems [4]. With all of that purpose, this species has a real potential for forest plantation development.

The main problem that often occurs in forestry nursery is a pest and disease attacks. The diseases that most frequently occur in the forestry nursery are dieback, leaf spot, and leaf blight [5]. Recently, there is a pathogen that has many hosts to any plants, which is *Botryodiplodia* sp., causing several tree
diseases, especially in the tropics regime [6]. According to Anggraeni and Lelana [7], Botryodiplodia sp. was reported as a pathogen of some forestry plants in Indonesia, causing leaf spots on Alstonia sp., Intsia bijuga Kuntze., Rhizophora mucronata Lamk., Macaranga gigantean Muell., root rot on Shorea sp., and stem disease on Aquilaria malaccensis Lamk. Disease inhibits the regeneration of plants at the classification of seedlings so that it will affect the quantity and quality of the plant. Furthermore, Saeed et al. [8] explained that dieback disease potentially causes a high mortality rate of the early stage of plant all over the world.

Based on the description above, research on the pathogenicity of M. eminii seedling against B. theobromae attack is essential. Therefore, this study aimed to examine the pathogenicity of B. theobromae on M. eminii leaves, to test that it can cause disease symptom. This information is essential for the next research based on diseases mechanism.

2. Method

2.1. Materials

The materials that used in this study consisted of labels, brushes, cotton buds, aluminum foil, labels, plastic wrap, Laminar Air Flow Cabinet (LAFC), autoclave, camera, 1 kg plastic, oven, Erlenmeyer, Petri dish, Bunsen, ½ kg plastic, cork borer, stationery, counting tools, sprayer, glass bottles, toolboxes and microscopes, carborundum, aquades, 75% alcohol, chloramphenicol, methylated spirits, PDA (Potato Dextrose Agar) and PDB (Potato Dextrose Broth) media. Living materials used consisted of 2.5 months old M. eminii seedlings and B. theobromae isolates.

2.2. Procedure

2.2.1. Pathogenicity test

Pathogenicity test parameters were disease incidence and dieback fungus attack intensity to the seedlings. Incidence of disease was determined as follow Ahmad et al [9]:

\[
\text{Disease Incidence (DI)} = \frac{n \times 100}{N}
\]

DI = disease incidence (%)

\(n\) = number of infected plant

\(N\) = number of plant sample observed

Disease intensity was calculated by following Townsend and Heurbergers also described in Stević et al. [10]:

\[
\text{DS} = \sum\left(\frac{n \times v}{N \times Z}\right) \times 100\%
\]

DS = disease severity (%)

\(N\) = number of leaf in every category

\(v\) = numeric number of every attack category

\(N\) = number of observed plant

\(Z\) = numeric number of the highest attack category

Disease severity scale and the numeric value of disease used were based on Aisah [11].
2.2.2. Experimental design

The experiments were arranged in a completely randomized design plot that was repeated five times each treatment. Four treatments were observed, namely treatment without injury and inoculation (S0), wound (S1), wound + inoculation (S2) and inoculation (S3).

The study used a completely randomized design (CRD) model Mattjik et al. [12].

\[ Y_{ij} = \mu + \tau_i + e_{ij} \]

Where \( Y_{ij} \) is observations on the i-th treatment, j-th test, \( \mu \) is average, \( \tau_i \) is impact of i-th treatment and \( e_{ij} \) is a random effect on the i-th test j test.

Furthermore, the variance test was performed. The treatment that significantly affected the observed parameters was followed by a multiple range test (Duncan's multiple range test-DMRT) with SAS 9.0 software [13].

2.2.3. Culture inoculation

\( B. \) theobromae isolates in PDB media aged 20 days were inoculated on each leaf of \( M. \) eminii treated with inoculation, using a sterilized brush. The inoculation was carried out at 16.00. Before being applied, the leaves are sterilized using 70% alcohol and then cleaned using distilled water. The leaves were wounded by using carborundum. Thus, the carborundum applied to the leaves by using a cotton bud.

2.2.4. Incubation

The treatment unit was placed in the Forest Pathology Greenhouse, IPB University. The size of the place used in the observation is 2 x 3 m, the light intensity of 65%. Each seedling was covered by using plastic, and this was intended to avoid contamination and spread of pathogens out.

2.2.5. Temperature and Humidity Measurement

Measurement of temperature and humidity is done every morning (07.00-08.00), afternoon (12.00-13.00) and evening (16.00-17.00). The humidity and temperature were calculated by using a hygrometer (Aisah et al. 2017).

2.2.6. Identification of Fungi

Identification was carried out from isolates that re-isolated from the symptomatic leaves in PDA and symptomatic leaves. Identification of fungi was carried out based on macroscopic and microscopic morphological characters. Macroscopic identification can be seen from the development of isolates on PDA. Microscopic identification was used as an optical lab viewer microscope. The measurement that used was a Raster image.

3. Result and discussion

The treatment of \( B. \) theobromae inoculation on \( M. \) eminii leaves showed the symptoms of blackish-brown spots (Figure 1). The symptoms of this \( M. \) eminii leaf are called leaf spot. Leaf spots on the leaves are death (necrotic) tissue which has strict-clear boundaries. These are the result of the infection caused by the pathogen (Munster 2018).
The results of disease severity for 30 days were an average of 0% for S0 treatment, 0% for S1, 27.56% for S2, and 23.57% for S3 treatments. The highest average occurred in the S2 treatment. The development of disease severity began to be seen on the first day of observation for the S3 treatment at 3.53% and the S2 treatment at 4.67%. The severity of the disease at the end of the observation for S2 treatment was 44.00%, and S3 treatment was 36.47%. In general, M. eminii leaves are categorized as somewhat moderate vulnerable to attack from B. theobromae because they are in the range of 16-50% (Table 1).

![Figure 1. M. eminii leaves a) healthy, b) symptomatic disease (red arrows)](image)

The results of disease incidence for 30 days were an average of 0% for S0, 0% for S1, 77.84% for S3, and 82.67% for S2 treatments. The most significant average was S2 treatment. Thus, the disease incidence those seen on the first day of observation on S2 and S3 treatments was 23.33% and 17.65%, respectively. At the end of observation of disease incidence for the treatment of S2 and S3 were 96.67% and 91.18%, respectively. The disease incidence for the treatments of S0 and S1 (wounded) was 0% from the beginning to the end. The treatment that showed the disease incidence was the leaf that given of B. theobromae inoculation.

Analysis of variance showed that the inoculation treatment of B. theobromae of M. eminii leaves significantly affected the incidence and severity of the disease. Meanwhile, Duncan’s multiple range tests of S0 and S1 treatments were not significantly different, but S2 and S3 treatments were significantly different (Table 2).

| Code | Disease level (%) | Plant resistance level to Infection |
|------|-------------------|------------------------------------|
| 0    | 0                 | Very resistance                     |
| 1    | 1 – 5             | Resistance                          |
| 2    | 6 – 15            | Tolerant                           |
| 3    | 15 – 50           | Moderate                           |
| 4    | 50 – 75           | Susceptible                        |
| 5    | >75               | Very susceptible                   |

Ex: Number followed by different alphabet showed the sign of significantly different at a 5% of confidence interval based on Duncan test.

Macroscopic observations on PDA media showed that the isolates were white up to 7 days old and gray to dark black in the old mycelium. The mycelium colony has a soft cotton texture with thick air mycelium, and the colony spreads from the center, irregularly. Isolates had rapid radial growth in Petri dishes. They had filled the Petri dishes (Ø = 9cm) in 4th day (Figure 2). Based on Yanti et al. (2018), B.
*theobromae* has a fluffy texture with thick air mycelium and colonies that spread from the middle with irregular topography (*rugose*). The isolates have fast radial growth that meets Petri dishes (Ø = 9 cm) after 3-4 days incubation period.

![Figure 2](image)

**Figure 2.** The macroscopic character of fungus isolates obtained from symptomatic *M. eminii* leaves on PDA media: a) young mycelium, b) old mycelium

Base on the microscopic symptom, the fungi have hyaline insulated hyphae. Meanwhile, the conidia are hyaline ellipsoids. The size is 18-20 x 10-13 μm (Figure 3). The figure showed the similarity characterization with *B. theobromae*. According to Winara (2014) states that hyphae from *B. theobromae* have the character of hyphae insulated, hyaline when young and greenish-brown when old. Conidia are hyaline ellipsoids when young, measuring 12.5 x 9.5μm, septate, dark brown when ripe, thin-walled and measuring 14.6 to 23.3 μm x 8.0 to 12.3 μm. According to Yanti *et al.* (2018), Mycelium *B. theobromae* has insulated hyphae, branching, hyaline when young, brown when old, and measuring 53-57 x 2-3 μm.

![Figure 3](image)

**Figure 3.** Microscopic characters of isolates obtained from symptomatic leaves disease: a) young conidia, b) hyaline hyphae

The environmental conditions essential factors for the development of a disease are the temperature and air humidity. Thus, the means of temperature recorded during 30 days of observation were 30.1°C. Means of air humidity recorded during 30 days observation were 70%. During 30 days of observation, *M. eminii* seedlings were in the optimum temperature, so the symptom occurred was caused by the biotic factors. 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 (Yanti *et al.* 2018). Finally, Cristin (2013) state that 55°C tested for 30 minutes is useful in stopping the growth of *B. theobromae*. 
4. Conclusion

Botryodiplodiataeobromae can attack Maesopsis eminii Engl. seedlings by infection without or with leaf injury. The symptoms that occur are leaf spot. Based on DMRT, wounded + inoculation had the most significant influence on disease severity and disease incidence, with average disease severity of 27.56% and disease incidence of 82.67%, respectively. The characterization of symptomatic leaves shows that the disease caused by B. theobromae.

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