Asian Journal of Plant Pathology, 2014
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2014.

Pathogenicity of *Botryodiplodia* sp. on the Seedling and Growth Characterization of Jabon (*Anthocephalus cadamba* (Roxb.) Miq.)

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ABSTRACT

Jabon (*Anthocephalus cadamba* (Roxb.) Miq.), also known as kadam, is among fast-growing tree species and has been considered as one of the promising plantation commodities. This study aimed to investigate the disease incidence and disease severity of jabon dieback in seedling as affected by pH and shaking intensity of *Botryodiplodia* sp. medium. Results revealed that the growth of the fungi was inhibited at pH 2 as shown by the diameter of colony formed and the much lower biomass as compared to control. Shaking intensity at 50 rpm showed the highest mycelial biomass of *Botryodiplodia* sp., followed by that of 100, 150 and 0 rpm. The dieback disease of jabon seedlings at all ages (3-5 months) only occurred in the treatment with inoculation with *Botryodiplodia* sp., fungal pathogen while the control treatment (no inoculation) did not show any symptom of the disease. The severity of dieback disease on jabon seedling was highest at 3 months old and less at other ages.

Key words: Jabon, dieback disease, *Botryodiplodia*, seedling

INTRODUCTION

Forest plantation development in Indonesia, either as community forest or industrial plantation forest is a response to the reduced natural forest in which the policy is expected to improve land productivity and environmental quality concomitantly. Accordingly, selection of tree species in the plantation is important for sustaining the development of the forest. Such selection should consider a number of factors, more importantly among others, i.e., grow fast with minimum nutritional requirements, can be used for wood processing industries and have a broad market, either internationally or nationally.

Jabon (*Anthocephalus cadamba* (Roxb.) Miq.), also known as kadam, is a tropical tree species that native to South Asia and Southeast Asia, including Indonesia. It is among fast-growing tree species and therefore has been considered as one of the promising plantation commodities (Kallio *et al*., 2011; Darmawan *et al*., 2013). The species can also be used for other purposes such as for post-mining land reclamation, reforestation and used as shade trees (Krisnawati *et al*., 2011). Due to these advantages, jabon has a high market demand in a large scale and therefore requires the availability of the seeds in sufficient quantities. Jabon is relatively resistant to disease once it is established but not so at the time of seedling in the nursery since the seedling is generally

susceptible to pathogen attack. This pathogen attack is one of the main causes of the reduced number of seed and lowered seedling quality of jabon. When jabon seedling is infected by pathogen, the leaves close to the infected branches turn yellow, then the damage continues to extend among the branches, the stems turn brown and then eventually become black. After macroscopic and microscopic observations, the identified pathogen is a fungus of the genus *Botryodiplodia* sp. resulting in dieback disease of the seedling (Aisah, 2014), although other fungal species may also exist.

The growth of *Botryodiplodia* sp., like other fungal species, is influenced by a number of environmental factors such as pH, oxygen concentration, temperature, nutrients, etc. Manipulation of such environmental factors could be an effective approach to control the growth of *Botryodiplodia* sp. in order to avoid or at least to minimize the impact of the fungal infection on jabon seedling. However, to date, the effects of pH and oxygen concentration on the growth of *Botryodiplodia* sp. have not been previously investigated. Therefore, the objective of the present study was to investigate the disease incidence and disease severity of jabon dieback in seedling as affected by pH and shaking intensity (influence the oxygen concentration) of *Botryodiplodia* sp. medium.

MATERIALS AND METHODS

Experimental materials: The materials used in this study were jabon (*A. cadamba*) seedlings age 3, 4 and 5 months old, obtained from Karya Barokah nursery in Bogor, Indonesia. The selected seedlings were uniform in size and in good condition. After being transported from the nursery, seedlings were acclimatized into the new environment for 3 days in order to prevent stress. Watering of the seedlings was conducted twice a day throughout the experimental period. Isolate of *Botryodiplodia* sp. was obtained from the Laboratory of Pathology, Department of Silviculture, Faculty of Forestry, Bogor Agricultural University, Indonesia. The isolate was isolated from jabon seedling that showed symptoms of the disease.

Medium used for growing of *Botryodiplodia* sp. *in vitro* was Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB). Briefly, medium was made by combining 200 g potato and 1 L distilled water and boiled subsequently until the potato was soft. The extract was separated and added up to 1 L of distilled water. Further, the extract was poured into a container containing 20 g dextrose (for PDB) or 15 g agar (for PDA). Preparation of PDA or PDB at various pH levels was performed by using the same materials and tools and additionally, HCl or NaOH were used to adjust the pH to become 2, 4, 6 or 8. The medium was sterilized by using an autoclave for 15 min at a temperature of 121°C and a pressure of 1 atm.

Growth characterization of *Botryodiplodia* sp.: The growth of *Botryodiplodia* sp. was characterized both in PDA and PDB media. The PDA medium was used to measure the growth characteristics of *Botryodiplodia* sp. at various pH levels. A piece of *Botryodiplodia* sp., colony was cut in a laminar air flow using a cork borer (0.8 cm diameter), planted right in the middle of a petri dish with a diameter of 9.0 cm. The treatments were PDA media with various pH levels, i.e., 6.8 (control), 2, 4, 6 and 8. Observations were made every 24 h by measuring the diameter of mycelial colony grown in the petri dish. The treatments were arranged in a Completely Randomized Design (CRD) that was repeated three times. The PDB medium was used to determine the biomass yield when *Botryodiplodia* sp. grown. Similar to the PDA medium, five different pH levels were set at 6.25 (control), 2, 4, 6 and 8. The fungal species was allowed to grow for six days. After six days,

the mycelia of *Botryodiplodia* sp. was separated from the media by using a filter paper. Subsequently, the mycelia of *Botryodiplodia* sp. on the filter paper was oven-dried for 24 h at 60°C and therefore the dry weight of *Botryodiplodia* sp. could be obtained.

Determination of biomass growth of *Botryodiplodia* sp., on the influence of shaking intensity was performed in PDB medium. A piece of *Botryodiplodia* sp. colony was inserted into an erlenmeyer flask containing PDB medium. The shaking intensity was tested at four levels, i.e., 0, 50, 100 and 150 rpm. The fungal species was grown for six days. After six days, the mycelia of *Botryodiplodia* sp. was separated from the media by using a filter paper. The mycelia of *Botryodiplodia* sp. on the filter paper was then oven-dried for 24 h at 60°C to determine the dry weight of the fungal species.

Pathogenicity test of *Botryodiplodia* sp. on jabon seedling: The method used in the pathogenicity test was the attachment method. Jabon healthy stem was initially sprayed with alcohol to remove any contaminants that may attach to the surface of the jabon bark. The stem was subsequently wounded with a sterile knife and then *Botryodiplodia* sp. was affixed to it. Observations were made on the incidence and severity of dieback disease at each age level. The incidence of illness or disease incidence is the percentage of plants attacked by pathogens (n) of the total plants observed (N) regardless of the severity of the disease. The equation used is:

$$\text{Incidence of disease (\%)} = n/N \times 100$$

Observation of disease severity due to fungal attack on the plant was calculated by using the following equation:

$$\text{Severity of disease (\%)} = \frac{\sum n \times V}{Z \times N} \times 100$$

where, n is the number of plants in each category, V is the score of each attack category, Z is the category of attack with the highest score and N is the total number of plants observed. The score of attack category is presented in Fig. 1.

Statistical analysis: Data obtained was analyzed by Analysis of Variance (ANOVA). Growth characterization of *Botryodiplodia* sp. employed the completely randomized design with three replicates. The statistical model was as follow:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where, Y_{ij} is the dependent variable at i-th treatment and j-th replicate, μ is the average value, α_i is the i-th treatment (pH or shaking intensity of the medium) and ε_{ij} is the experimental error.

The pathogenicity test was arranged in a split plot design in three replicates. The main plot was the pathogenic fungi inoculation (*Botryodiplodia* sp.) and the subplot was jabon seedling age. The experimental unit was consisted of ten jabon seedlings each and the seedlings were planted in separated polybags. The first factor, i.e., pathogenic fungi inoculation consisted of two levels, namely control (without inoculation of pathogenic fungi) and inoculation with *Botryodiplodia* sp. The second factor was the seedling age, consisted of three levels, i.e., age of 3, 4 and 5 months. The statistical model was as follow:

Fig. 1(a-f): Score of attack category on jabon seedling: (a) 0: No. symptom at all, (b-c) 1: Plant is withered or ≤25% necrosis, (d) 2: 26-50% necrosis, (e) 3: >50% necrosis and (f) 4: Plant is dead

$$Y_{ijk} = \mu + \alpha_i + \delta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where, Y_{ijk} is the intensity of the attack on the k-th replicate at the i-th level of the inoculation factor and the j-th level of the age factor, μ is the average value of the attack intensity, α_i is the main effect of the i-th level of inoculation factor, δ_{ik} is the effect of error that occur in the i-th level of inoculation factor in the k-th replicate (main plot error), β_j is the main effect of the j-th level of the age factor, $(\alpha\beta)_{ij}$ is effect of interaction with the i-th level of inoculation factor and the j-th level of the age factor and ε_{ijk} is the effect of the error on the k-th replicate at the i-th inoculation factor and the j-th age factor (subplot error). When the ANOVA results showed significantly different at $p<0.05$, the analysis was continued with Duncan's multiple range test.

RESULTS AND DISCUSSION

An example of a normal healthy jabon seedling and a jabon seedling infected with *Botryodiplodia* sp. is shown in Fig. 2. After being infected with the pathogenic fungi, the symptoms were observed starting from the closest leaf to the inoculation site with the pathogenic isolates. The color of the leaves was changed from yellowish to brownish and then the damage was extended to other parts. When the infection has been spread to the bud and stem, the entire plant is blackened and dead.

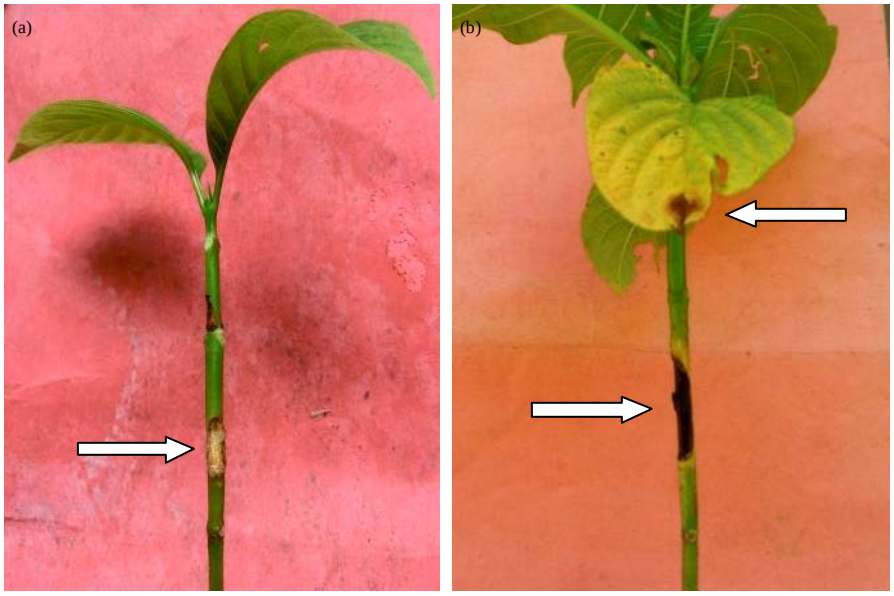

Fig. 2(a-b): Normal healthy jabon seedling and a jabon seedling infected with *Botryodiplodia* sp.

Table 1: Diameter of *Botryodiplodia* sp. colony during 3 days period as influenced by various pH

	Botryodiplodia sp. colony diameter (cm)		
Medium pH	Day 1	Day 2	Day 3
Control (6.8)	3.0	7.5	9.0
2.0	0.0	0.0	0.0
4.0	2.5	7.4	9.0
6.0	2.5	7.1	9.0
8.0	2.4	6.4	9.0

Based on the *in vitro* growth testing of *Botryodiplodia* sp., the growth of the fungi was inhibited at pH 2 as shown by the diameter of colony formed (Table 1) and the much lower biomass as compared to control (Fig. 3a). Other medium pH (4, 6 and 8) did not show any difference in biomass of *Botryodiplodia* sp. than that of control, showing the fungi had no limitations to grow within the range of pH. Fungi generally have the ability to survive in a wide range of pH, i.e., between 3-9 (Tarr, 1972; Saha *et al*., 2008). At a pH lower than such range, apparently the fungi species is unable to grow optimally. Fungi are heterotrophs that obtain nutrients through absorption of small organic molecules from the surrounding medium by secreting various hydrolytic enzymes and the enzymes cannot be active at extremes pH which explain the low *Botryodiplodia* sp. growth at pH 2. Shaking intensity at 50 rpm showed the highest mycelial biomass, followed by that of 100, 150 and 0 rpm (no shaking at all; Fig. 3b). This may suggest that *Botryodiplodia* sp. grow better with a certain rate of shaking intensity since the majority of plant pathogens are aerobic and therefore the supply of oxygen is necessary for optimum growth (Tarr, 1972).

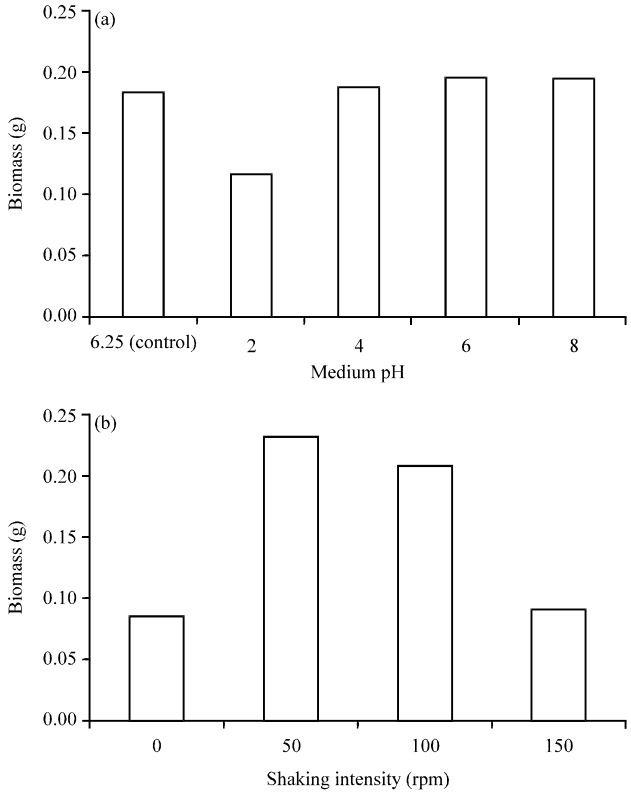

Fig. 3(a-b): Influence of (a) pH and (b) Shaking intensity of medium on biomass of *Botryodiplodia* sp.

Table 2: Disease incidence and severity of jabon seedling at different age either without or with pre-inoculated *Botryodiplodia* sp.

Fungal inoculation	Age (months)	Incidence (%)	Severity (%)
No	3	0[a]	0[a]
No	4	0[a]	0[a]
No	5	0[a]	0[a]
Yes	3	100[b]	61[d]
Yes	4	100[b]	42[b]
Yes	5	100[b]	54[c]

Different superscripts within the same column are significantly different at $p<0.05$ as tested by ANOVA and the subsequent Duncan's multiple range test

The dieback disease of jabon seedlings at all ages (3-5 months) only occurred in the treatment with inoculation with *Botryodiplodia* sp. fungal pathogen while the control treatment (no inoculation) did not show any symptom of the disease (Table 2). This proved that the fungal species is pathogenic and has the ability to cause the disease (virulent). The dieback disease due to *Botryodiplodia* sp. was reported in the area of cocoa plantations in Cameroon which limited the cocoa production in the country (Mbenoun *et al*., 2008). The fungal pathogen was also found in rubber trees in Vietnam and caused dieback in nursery and resulted in a serious suppression of rubber production (Pha *et al*., 2009). Development of a disease is supported by three factors, namely susceptible host, virulent pathogen and supportive environment. Jabon seedlings

apparently are very susceptible to the infection. With regard to the pathogen, according to Semangun (2007), *Botryodiplodia* sp. is a fungus that has a wide range of host. This pathogen is a weak parasite and the infection is done through mechanical injury as a result of cuts or wounds caused by insects. With regard to environment, the condition which is favorable for pathogen to proliferate is high moisture, abundant nutrients and high temperature; such conditions are typically occurred in Bogor and hence stimulate the pathogen to germinate and to penetrate into plant tissues. Wound is a way of pathogen entry; pathogen penetration is faster with the wound. It was evident from the present study that all jabon seedlings (100%) showed symptoms of the disease after being wounded and inoculated. The seedlings without inoculation showed no symptoms at all although they were cut wounded beforehand.

The severity of dieback disease on jabon seedling was highest at 3 months old. The severity of the seedling at 4 and 5 months old was 42 and 54%, respectively. Uninoculated jabon seedling showed 0% disease severity since there was no symptoms of the disease. Disease severity is related to defense mechanism of the plant against the pathogen. In general, the plant provides a response to pathogen attack and the response is responsible for the plant resistance to pathogen. There are two ways of plant defense mechanisms, firstly with the structural properties of the plant that serves as a physical barrier and will inhibit pathogens to enter and spread within the plant cell and secondly, biochemical response in the form of chemical reactions occurring within the cell and plant tissue (Groenewald, 2005).With regard to the later, according to Agrios (1997), biochemical resistance in plants is determined by the availability of nutrients for pathogens and the compounds that are toxic to the pathogens. Such compounds include proteins, sugars and polyphenols in which their concentrations may vary according to age and surrounding environment. Severity of the disease is depended on plant species. For instance, in tomato plants, the older the plant, the higher the severity of the disease (Sumaraw, 1999) and the opposite for the pine trees in which the resistance to pathogen attack increased when the seedlings are getting older (Achmad *et al*., 2012). Based on these results, it is recommended to select the 4 month old seedling as planting material.

CONCLUSION

The growth of *Botryodiplodia* sp. was best at pH 4. At pH 2, the growth of the fungal species was hampered. Mycelia biomass was highest at 50 rpm shaking intensity. In the pathogenicity test of *Botryodiplodia* sp. on jabon seedlings at different age, the symptoms of dieback disease occurred only in the pathogen pre-inoculated seedlings and reached a value of 100% disease incidence. The lowest disease severity value was 42%, i.e. for jabon seedlings at the age of 4 months. The seedlings without inoculation of pathogenic fungi showed no symptoms of disease until the end of the observation period.

REFERENCES

Achmad, S.H., S. Harran, E.G. Said, B. Satiawiharja and M.K. Kardin, 2012. Mechanism of lodoh pathogen infection on *Pinus merkusii*. J. Trop. Silvicult. Sci. Technol., 3: 57-64.

Agrios, G.N., 1997. Plant Pathology. 4th Edn., Academic Press, San Diego, CA, USA., Pages: 635.

Aisah, A.I., 2014. Identification and pathogenicity of primary fungi causing dieback disease on jabon seedlings (*Anthocephalus cadamba* Miq). Master Thesis, Bogor Agricultural University, Indonesia.

Darmawan, W., D. Nandika, I. Rahayu, M. Fournier and R. Marchal, 2013. Determination of juvenile and mature transition ring for fast growing sengon and jabon wood. J. Indian Acad. Wood Sci., 10: 39-47.

Groenewald, S., 2005. Biology, pathogenecity and diversity of *Fusarium oxysporum* f.sp. cubense. Master Thesis, University of Pretoria, South Africa.

Kallio, M.H., M. Kanninen and D. Rohadi, 2011. Farmers' tree planting activity in Indonesia: Case studies in the provinces of central JAVA, Riau and South Kalimantan. For. Trees Livelihoods, 20: 191-209.

Krisnawati, H., M. Kallio and M. Kanninen, 2011. *Anthocephalus Cadamba* Miq.: Ecology, Silviculture and Productivity. CIFOR Publisher, Bogor, Indonesia, ISBN-13: 9786028693387, Pages: 11.

Mbenoun, M., E.H.M. Zeutsa, G. Samuels, F.N. Amougou and S. Nyasse, 2008. Dieback due to *Lasiodiplodia theobromae*, a new constraint to cocoa production in Cameroon. Plant Pathol., 57: 381-381.

Pha, T.A., P.T. Dung, N.D. Hieu and N.A. Nghia, 2009. Disease caused by *Botryodiplodia theobromae* Pat. on rubber tree in Vietnam. Rubber Research Institute of Vietnam, Ho Chi Minh, Vietnam.

Saha, A., P. Mandal, S. Dasgupta and D. Saha, 2008. Influence of culture media and environmental factors on mycelial growth and sporulation of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. J. Environ. Biol., 29: 407-410.

Semangun, H., 2007. Diseases of Horticultural Plants in Indonesia. Gadjah Mada University Press, Yogyakarta, Indonesia.

Sumaraw, S.M., 1999. [Critical period of tomato to *Alternaria solani* (Ell. and G. Martin) Sor. infection and its determinant]. Bull. Plant Pest Dis., 11: 67-72, (In Indonesian).

Tarr, S.A.J., 1972. The Principles of Plant Pathology. Macmillan Publishing, London, UK., ISBN-13: 9780876910696, Pages: 400.