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Identification and characterization of *Pestalotiopsis* spp. causing leaf spot and leaf blight on jabon (*Neolamarckia* spp.) in Indonesia

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Abstract. *Herliyana EN, Oktavianto P, Siregar UJ. 2022. Identification and characterization of* Pestalotiopsis *spp. causing leaf spot and leaf blight on jabon (*Neolamarckia *spp.) in Indonesia. Biodiversitas 23: 6547-6556.* Jabon (*Neolamarckia* spp.) is one of the fast-growing trees with high economic value for industrial plantations. Its monoculture planting system is faced with certain problems, such as the leaf spot and leaf blight diseases caused by *Pestalotiopsis* sp. Therefore, the aim of this research was to describe the morphology of *Pestalotiopsis* species through macroscopic and microscopic observations, followed by the identification of fungus using DNA barcoding causing leaf spot and leaf blight on the tree. A total of 11 *Pestalotiopsis* isolates were used for this research. The morphology was identified by observing mycelium colonies and fungal conidia, while DNA barcoding was initiated after DNA isolation using cetyltrimethylammonium bromide (CTAB) method, amplification with ITS 1 and 4 primers, followed by sequencing process. The results of DNA barcoding showed that 5 species of *Pestalotiopsis*, namely *Pseudopestalotiopsis theae*, *Pestalotiopsis microspora*, *Pestalotiopsis palmarum*, *Neopestalotiopsis clavispora*, and *Pestalotiopsis virgatula* were found associated with leaf spot and blight disease. All the five species were further classified into 3 clades using phylogeny according to their median cells and apical appendage shape. The clade X consisted of *P. palmarum*, *N. clavispora*, and *P. virgatula* with versicolorous median cells, whereas clade Y included *P. microspora* with concolorous median cell, and clade Z consisted *Ps. theae* with concolorous median cell and knobbed apical appendage.

Keywords: Identification, jabon, leaf spot, *Pestalotiopsis*, phylogeny analysis

INTRODUCTION

Wood is one of the important products produced from the forest and serves as a source of revenue for the country. Its continuous production due to high demand has led to an annual increase in the need for timber. Natural forest does not have the capacity to sustain the supply of wood, and this means there is a need for industrial plantations to increase wood production capacity. A particular example of plants that can be used for this purpose is jabon (*Neolamarckia* spp.) and the production of its log was reported to have increased from 4,999.98 m3 in 2013 (BPS 2013) to 19,606.41 m3 in 2016 (BPS 2016).

Jabon is one of the trees with high economic value usually used for industrial plantation it is a fast-growing species with high adaptability and easy silvicultural treatment. It has two species which include the red jabon (*Neolamarckia macrophylla* (Roxb.) Bosser) and white jabon (*Neolamarckia cadamba* (Roxb.) Bosser). Jabon grows well on fertile and well-aerated soils and germinates efficiently at temperatures of 15.5-32°C and average annual rainfall of 1,500-5,000 mm in its natural habitat but also has the ability to grow in arid areas with an annual rainfall of 200 mm. The tree also grows well at an altitude of 300-800 meters above sea level and its wood can be used as raw material for plywood, light construction, floor, pulp, paper, boxes, simple tools, and roadside decoration (Krisnawati et al. 2011).

The monoculture plantation of this tree is usually faced with the problem of pest and disease attacks which have the ability to reduce its productivity. Diseases like leaf spot and leaf blight and some fungi can also cause defoliation of some or all of the leaves. Diseased leaves impair the ability to reduce the rate of photosynthesis and cause suboptimal growth or even death. It has been previously reported that leaf disease in the nursery can lead to decline in seedling quality and death (Irawan et al. 2015). Jabon leaf blight and leaf spot is caused by *Pestalotiopsis* sp., which belongs to the Ascomycota division, Sordariomycetes class, Xylariomycetidae subclass, Xylariales order. This fungus is divided into 3 genera based on the color of median cells and the sequence data and these include the *Pestalotiopsis*, *Pseudopestalotiopsis*, and *Neopestalotiopsis*. It is also important to note that the fungus also has the ability to infect a range of hosts (Hertz 2016) and reduce the production and economic value of plants (Vasic et al. 2017). However, the monoculture planting of jabon is increasingly widespread and there is a possibility of its production to be endangered by a higher risk of disease attack. This means it is necessary to identify the invading pathogen in order to determine its potential and control technique because *Pestalotiopsis* is a complex fungus with diverse morphological characteristics in the same species (Espinoza et al. 2008) which makes its identification through morphology to be difficult. Therefore, it is possible to apply DNA barcoding in identifying the pathogens

accurately and to analyze the phylogenetic relationship between species.

It is also important to note that information about *Pestlaotiopsis* is not widely available in Indonesia, therefore, there is a need for molecular identification and morphological characterization in order to create an accurate database. The aim of this research was to identify and describe the morphology of *Pestalotiopsis* species by macroscopic and microscopic observations, followed by identification of pathogen using DNA barcoding that causes leaf spot and leaf blight on jabon.

MATERIALS AND METHODS

Research area

The sampling was conducted at Sukabumi, Bogor, Pandeglang, and Kendari such that the *Pestalotiopsis* for red jabon were isolated from Kendari and those for white jabon are from Sukabumi, Bogor, and Pandeglang, Indonesia (Table 1). The samples were processed at the Forest Pathology and Forest Genetics Laboratory, Silviculture Department, Faculty of Forestry, IPB University. This research was conducted from January to June 2019.

Procedures

Symptomatology and isolation

The symptoms were characterized by spots and blight found on the leaves, with a focus on the shape, size, and color. The disease leaves were rinsed with alcohol and isolation was done on potato dextrose agar (PDA) media. The growing pathogen was later transferred to a new PDA media to obtain pure culture which was further incubated at room temperature for 14 days.

Morphology observation

Pestalotiopsis species grown on petri dishes were observed macroscopically and microscopically. The macroscopic aspect focused on the colony shape, color, texture, and mycelial rate growth which were measured using the average horizontal and vertical diameters. In microscopic characterization, 20 conidia were measured for the shape, size, median cell color, number and shape of apical appendage, as well as the length of the apical and basal appendage (Maharachchikumbura et al. 2011).

Molecular characterization

The molecular identification process was conducted through DNA extraction, DNA amplification, and sequencing. The extraction involved using the cetyltrimethylammonium bromide (CTAB) method described by Borges et al. (2009) with modification and the result was amplified using ITS 1 primer (5'-CTTGGTCATTTAGAGGAAGTA-A-3'), ITS-4R primer (5'-TCCTCCGCTTA TTGATATGC-3'), and MyTaq HS Redmix 2x. It is important to note that the result was diluted twice in nuclease-free water. The ingredients for amplification included 1 µL DNA template, 1 µL ITS1 primer, 1 µL ITS-4R primer, 12.5 µL MyTaq HS Redmix 2x, and 9.5 µL Nuclease Free Water (NFW). The process involved one initial denaturation cycle at a temperature of 95°C for 1 minute followed by 30 cycles of denaturation at 95°C for 15 seconds, annealing at 58°C for 15 seconds, extension at 72°C for 10 seconds, and the final extension cycle at 72°C for 7 minutes. The amplification results were electrophoresed on 2% agarose gel such that a total of 3 µL amplified DNA was inserted in the gel and electrophoresed at 100 volts for 30 minutes after which the results were placed on the UV transilluminator to view the DNA band. Further, the result of amplification was sent to PT. Genetika Science.

Data analysis

The *Pestalotiopsis* conidia were measured using Image Raster 3.0 software and the average results of growth and conidia were recorded. The sequencing data were processed using MEGA-X software. The results of DNA base sequences were also analyzed by comparing the values with the database in the NCBI (National Center for Biotechnology Information) using the Basic Local Alignment Search Tool (BLAST). Moreover, the genetic distance and phylogeny analysis were conducted using MEGA X software with maximum likelihood and bootstrap 1,000 methods.

RESULTS AND DISCUSSION

Symptoms on jabon leaf

The symptoms associated with the attack of *Pestalotiopsis* on jabon leaves included leaf spot and leaf blight, which cause changes in the color of leaves. B4 isolates showed parallel light green color spot with a diameter of 0.2 cm. B5 and S7 isolates had light brown color in the middle and dark brown on the edges with 0.4-1.0 cm diameter, while B7 isolate showed black spots with 0.1-0.2 cm diameter. Moreover, B8, B9, and BS1 produced gray spots at the middle part of the leaf, edges were brown, yellow halo, and 0.2-1.3 cm in diameter, while HS4 produced brown blight area on the edge of leaf with a diameter of 2.2-2.4 × 0.5-1.0 cm (Figure 1).

Table 1. Collection sites of *Pestalotiopsis* spp. isolates derived from jabon tree

Isolates code	Locations	Hosts
B4, B5, B7, B8, B9	Kendari	Red jabon
BS1, HS4, S7	Sukabumi	White jabon
BP5, HP5	Pandeglang	White jabon
BG1	Bogor	White jabon

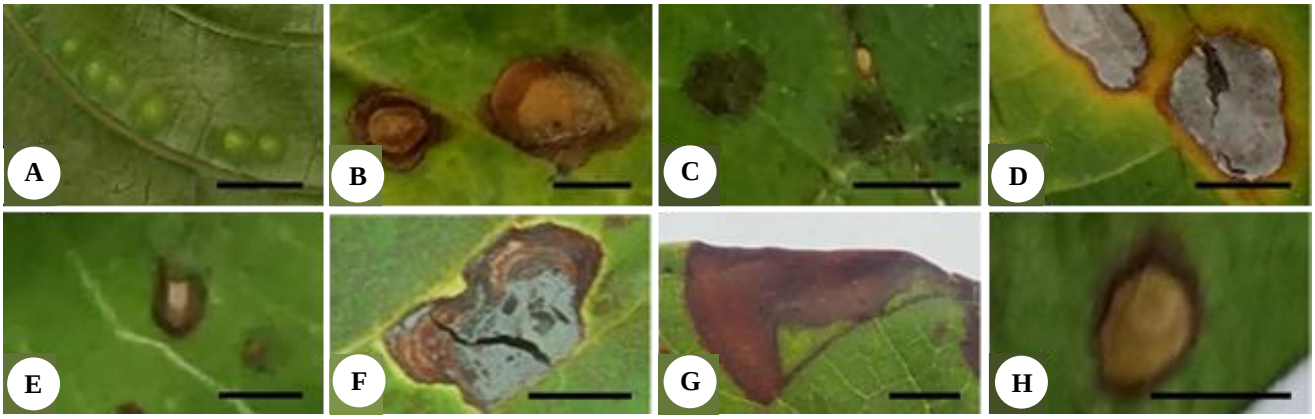

Figure 1. Symptoms on jabon leaf caused by *Pestalotiopsis*. A. B4, B. B5, C. B7, D. B8, E. B9, F. BS1, G. HS4, and H. S7 isolates (scales bar = 0.5 cm)

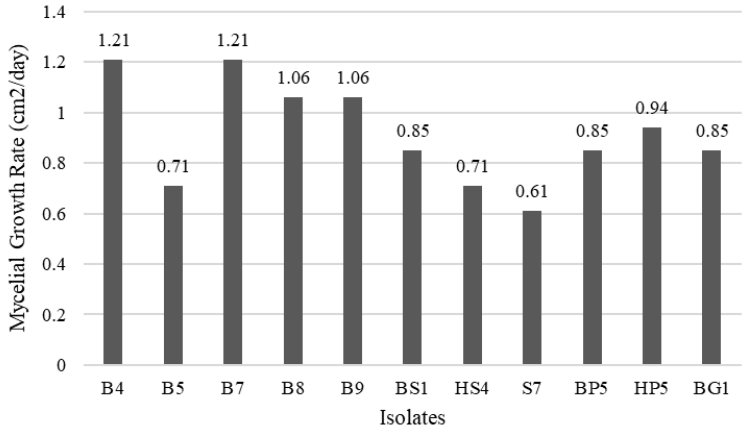

Figure 2. Average mycelium growth of 11 *Pestalotiopsis* isolates after 7 days on potato dextrose agar

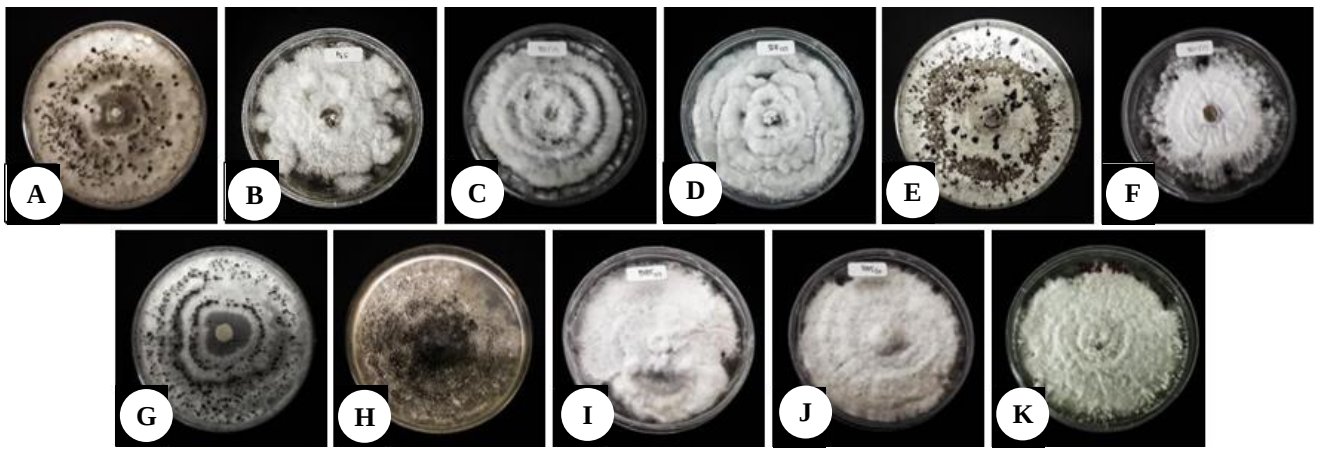

Figure 3. Colony morphology of *Pestalotiopsis* isolates on potato dextrose agar. A. B4, B. B5, C. B7, D. B8, E. B9, F. BS1, G. HS4, H. S7, I. BP5, J. HP5, and K. BG1 isolates

Mycelium colony characteristics and growth rate

The 11 isolates had different mycelium growth after 7 days, such as B4 and B7 had the fastest growth rates of 1.21 cm/day, followed by B8 and B9 with 1.06 cm/day, HP5 had 0.94 cm/day, BS1, BP5, and BG1 had 0.85 cm/day, B5 and HS4 0.71 cm/day, and S7 had the slowest with 0.61 cm/day, as indicated in Figure 2. The colony morphology was observed at the initial stage to determine fungal characteristics and results showed that morphologies of 11 *Pestalotiopsis* mycelium colonies were not much different from each other. The colonies were white, fine-textured like cotton, and formed a circular pattern with thick and thin mycelium. Some isolates, such as B4 and S7 were observed to have a brownish mycelium color. It was

also note that black or dark brown color acervuli started to grow between day 7 and 21 of incubation. Some acervuli were grow regularly found in HS4 isolate, while scattered randomly, such as in isolates B9 and B4, or even in the middle as in isolate S7, as presented in Figure 3.

Characteristics of *Pestalotiopsis* conidia

Pestalotiopsis conidia had 5 cells divided into apical cells, 3 median and basal cells, and had 4 septa. The conidia were fusiform, with straight or curved cell edges, and had flagellum at both ends. The flagellum in the apical was setula, and flagellum in the basal cell was pedicel. The median cells were yellow to brown, while apical and basal were hyaline. It was also observed that the median cell consists of concolorous and versicolorous median cells. It was important to note that there were 2-4 apical appendages with different forms and sizes (Figure 4).

The conidia characteristics of the 11 isolates were also observed to be differ, with BG1 recorded to have the longest with 30.40 µm, BS1 had the widest with 6.16 µm, while HP5 had the smallest dimensions with a length of 21.47 µm and a width of 5.18 µm. Based on the median cell color, B4, B8, B9, HS4, BP5, HP5, and BG1 were concolorous. Isolate B4, B8, B9, HS4 and BP5 had brown color median cell, while HP5 and BG1 isolates were light brown. Meanwhile, B5, B7, BS1, and S7 were versicolorous with the two upper median cells found to be darker than the lower one as presented in Table 2.

Based on the number of apical appendages, the findings showed that B4, B8, B9, BS1, and BP5 had 2-4 apical appendages, while B5, B7, HS4, S7, HP5, and BG1 had 2-3. It was also noted that BS1 isolate had the longest appendages with 30.80 µm, while B5 had the shortest with 8.12 µm. Moreover, B4, B8, B9, and HS4 had a knobbed like appendages, whereas B5, B7, BS1, BP5, HP5, and BG1 had tubular apical appendages. The longest basal appendage was found in BG1 with 7.8 µm and the shortest was in B7 with 4.0 µm (Table 2).

Molecular characterization and identification

The DNA amplification was conducted using ITS1 and ITS4 primers and results were estimated at 550 to 650 bp for all the isolates (Figure 5). The results obtained from 11 isolates also had a query cover value between 99-100% and means that they were similar to the *Pestalotiopsis* sequence in GenBank. Moreover, their E-value was 0, indicating that results were accurate because there were no errors in alignment between the input DNA sequence and in GenBank. These results showed that all the 11 isolates were identified as *Pestalotiopsis* species (Table 3). The result of BLAST-N showed that B4, B8, B9, and HS4 were identified as *Pseudopestalotiopsis theae*, B5 as *Pestalotiopsis palmarum*, B7 as *Neopestalotiopsis clavispora*, BG1 and BS1 as *Pestalotiopsis microspora*, and S7 as *Pestalotiopsis virgatula* (Table 3).

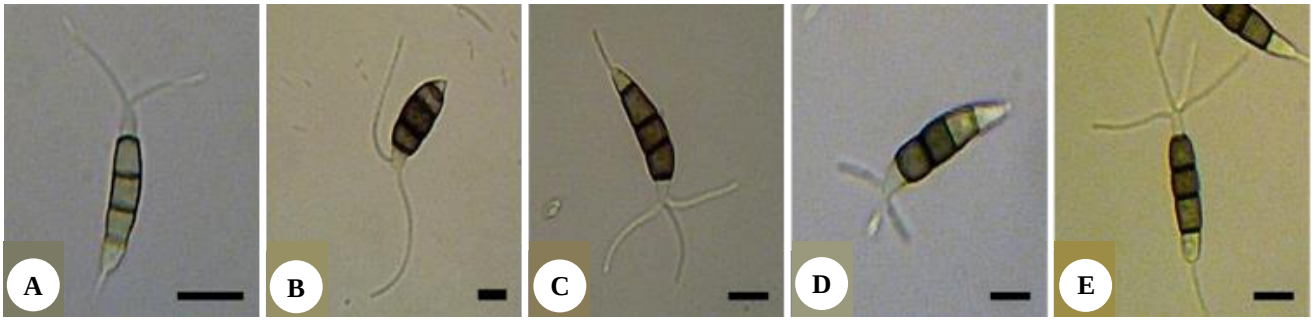

Figure 4. Characteristics of *Pestalotiopsis* conidia. A. Conidia with concolorous median cell and 2 apical appendages, (1. Setula, 2. Apical, 3. Basal, 4. Pedicel, 5. Median) B. Conidia with versicolorous median cell and 2 apical appendages, C. Conidia with concolorous median cell and 3 apical appendages, D. Conidia with versicolorous median cell and 3 apical appendages, E. Conidia with concolorous median cell and 4 apical appendages

Table 2. Characteristics of *Pestalotiopsis* conidia

Isolates	Length (µm)	Width (µm)	Median cell color	Number of apical appendages	Apical appendage length (µm)	Apical appendage form	Basal appendage length (µm)
B4	27.82	5.87	Concolourous, brown	2-4	23.74	Round	4.5
B5	23.51	5.90	Versicolorous	2-3	8.12	Cylinder	5.9
B7	26.56	5.78	Versicolorous	2-3	18.52	Cylinder	4.0
B8	28.93	5.53	Concolourous, brown	2-4	16.90	Round	5.8
B9	27.59	5.37	Concolourous, brown	2-4	12.85	Round	4.2
BS1	25.31	6.16	Versicolorous	2-4	30.80	Cylinder	6.5
HS4	24.61	5.55	Concolourous, brown	2-3	17.59	Round	5.5
S7	22.22	6.15	Versicolorous	2-3	15.59	Cylinder	3.4
BP5	26.33	5.40	Concolourous, brown	2-4	13.89	Cylinder	5.2
HP5	21.47	5.18	Concolorous, light brown	2-3	22.34	Cylinder	5.2
BG1	30.40	5.88	Concolorous, light brown	2-3	20.68	Cylinder	7.8

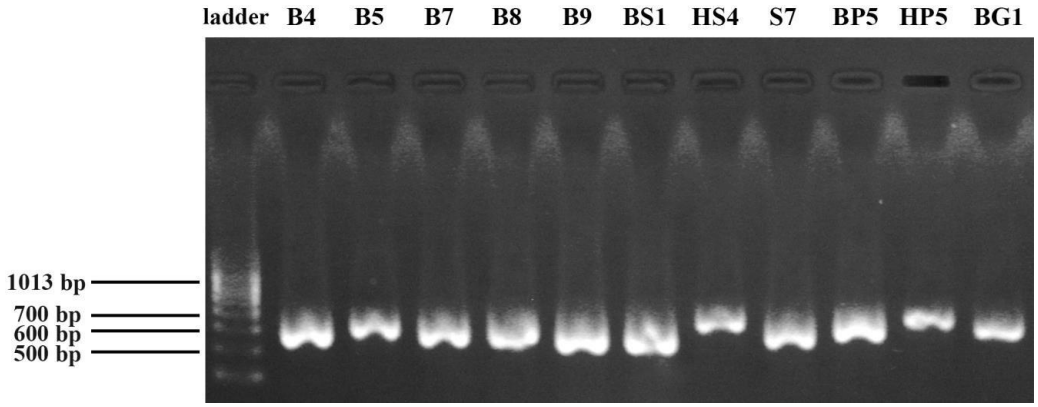

Figure 5. Amplification products and markers in agarose gel

Table 3. BLAST-N results of ITS sequence from *Pestalotiopsis*

Isolates	Species	Query cover	E value	% Identification	Accession no.
B4	*Pseudopestalotiopsis theae*	99 %	0	99.45 %	KM510412.1
B5	*Pestalotiopsis palmarum*	100 %	0	99.81 %	GQ888738.1
B7	*Neopestalotiopsis clavispora*	100 %	0	99.82 %	KR052094.1
B8	*Pseudopestalotiopsis theae*	100 %	0	99.82 %	KM510412.1
B9	*Pseudopestalotiopsis theae*	100 %	0	99.82 %	KM510412.1
BS1	*Pestalotiopsis microspora*	100 %	0	100 %	MK120574.1
HS4	*Pseudopestalotiopsis theae*	100 %	0	99.82 %	KM111476.1
S7	*Pestalotiopsis virgatula*	100 %	0	100 %	AY924281.1
BP5	*Pestalotiopsis microspora*	100 %	0	99.44 %	KT459349.1
HP5	*Pestalotiopsis microspora*	100 %	0	99.81 %	KT459349.1
BG1	*Pestalotiopsis microspora*	100 %	0	100 %	MK120574.1

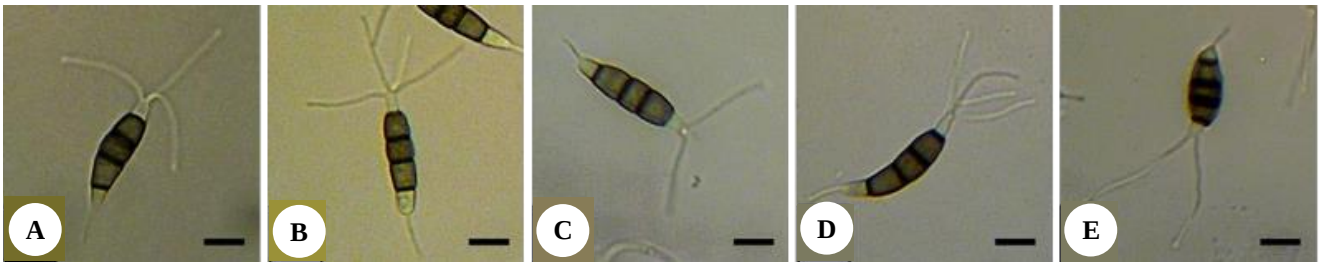

Figure 6. Conidia of *Ps. theae*. A-B. B4, C. B8, D. B9, E. HS4 (scales bar = 10 µm)

Pseudopestalotiopsis theae

Pseudopestalotiopsis theae had fusiform conidia and straight to slightly curved sides. The conidia had 5 cells, 4 septa, and 21.0-36.0 × 4.3-6.8 µm in diameter. It was also observed that it had doliiform, concolorous median cells, while septa were darker than the median cells. Moreover, the first median cell was 3.8-6.9 µm long, second was 3.7-6.5 µm, and the third was 2.5-8.1 µm. The apical cell was 3.2-6.7 µm long, hyaline, conic, with 2-4 knobbed apical appendages, arising from the apical crest, unbranched, filiform, and 9.0-28.4 µm long, while the basal cell was 2.4-6.8 µm long, conic, hyaline, with a single tubular basal appendage, 2.4-9.3 µm long, and unbranched (Figure 6).

Pestalotiopsis microspora

This species had fusiform conidia with straight to slightly curved sides. The conidia had 5 cells, 4 septa, and 18.1-35.9 × 4.4-6.9 µm in size. Median cells were doliiform and concolorous but sometimes versicolorous with darker septa. The first median cell was found to be 3.3-6.6 µm long, second was 3.3-5.7 µm, and third was 3.6-6.6 µm. In addition, the apical cell was 3.2-6.1 µm long, hyaline, conic, with 2-4 tubular apical appendages, arising from the apical crest, unbranched, filiform, and 11.2-35.4 µm long. The basal cell was 2.9-6.4 µm long, conic, hyaline, with a single tubular basal appendage, 3.5-10 µm long, and unbranched (Figure 7).

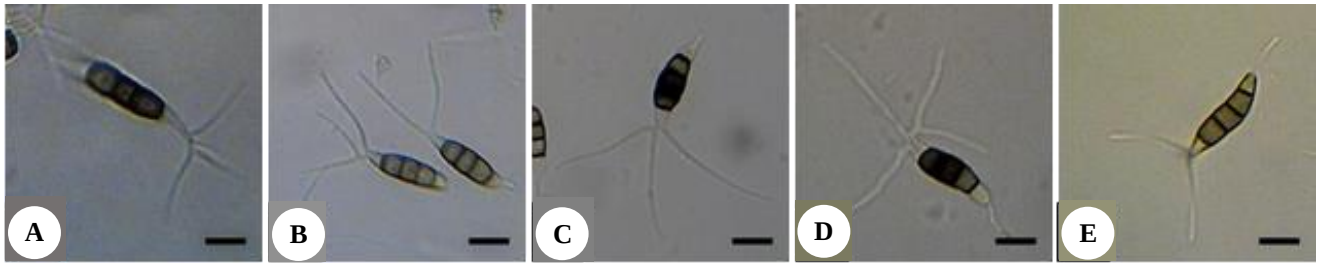

Figure 7. Conidia of *P. microspora*. A. BP5 isolate, B. HP5 isolate, C-D. BS1 isolate, E. BG1 isolate (scales bar = 10 µm)

Neopestalotiopsis clavispora

This species had fusiform conidia and straight to slightly curved sides. The conidia had 5 cells, 4 septa, and 22.0-30.5 × 5.1-6.7 µm in diameter. Moreover, the median cells were doliiform and versicolorous with the two upper ones being darker than the lower one, and the septa being darker than the median cells. The length of the first median cell was 3.9-6.0 µm, second was 4.2-6.1 µm, and the third was 4.1-6.7 µm. However, the apical cell was 4.1-5.7 µm long, hyaline, conic, with 2-4 tubular apical appendages, arising from the apical crest, unbranched, filiform, and 22.0-30.5 µm long. It was also observed that the basal cell was 4.1-6.5 µm long, conic, hyaline, with a single tubular basal appendage, 2.2-5.8 µm long, and unbranched (Figure 8).

Pestalotiopsis virgatula

This species also had fusiform conidia and straight to slightly curved sides. The conidia had 5 cells, 4 septa, and 18.1-25.4 × 5.9-6.9 µm in size. The median cells were doliiform and versicolorous with two upper ones found darker than the lower one and the darker septa than the median cells. The length of the first median cell was 3.9-5.0 µm, second was 3.5-5.0 µm, and third was 3.5-5.1 µm. The apical cell was 2.7-5.5 µm long, hyaline, conic, with 2-3 tubular apical appendages, arising from the apical crest, unbranched, filiform, and 13.9-17.7 µm long. The findings also showed that the basal cell was 2.7-4.8 µm long, conic, hyaline, with a single tubular basal appendage, 2.7-4.1 µm long, and unbranched (Figure 9).

Pestalotiopsis palmarum

This species had fusiform conidia, straight to slightly curved sides. Conidia had 5 cells, 4 septa, and sized 20.4-27.4 x 5.1-6.8 µm. Median cells was doliiform, versicolorous with the two upper median cells darker than the lower one, and septa darker than median cells. The first median cell 4.1 - 5.9 µm long, the second median cell 3.9-6.3 µm long, and the third median cell 3.7-6.7 µm long. Apical cell 3.0-5.6 µm long, hyaline, conic, with 2-3 tubular apical appendages, arising from apical crest, unbranched, filiform, and 6.1 - 10.8 µm long. Basal cell 3.1-6.1 µm long, conic, hyaline, with single tubular basal appendage, 4.7-7.5 µm long, and unbranched (Figure 10).

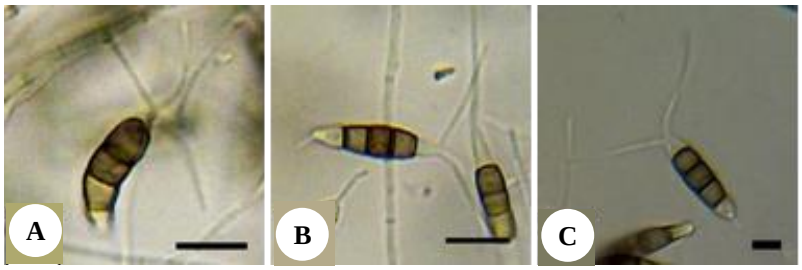

Figure 8. Conidia of *N. clavispora*. A-C. B7 isolate (scales bar = 10 µm)

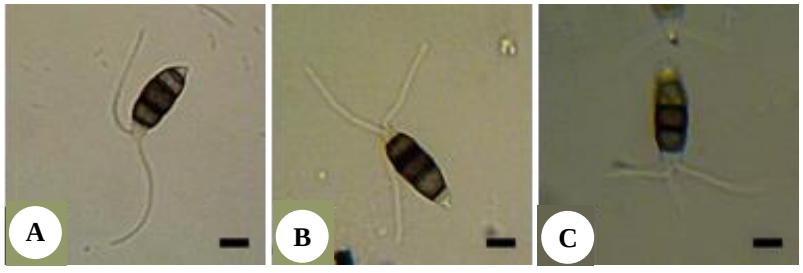

Figure 9. Conidia of *P. virgatula*. A-C. S7 isolate (scales bar = 10 µm)

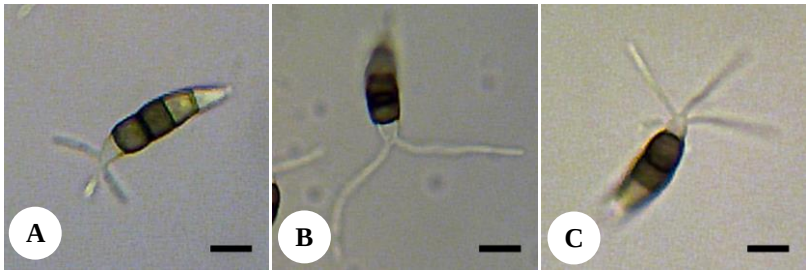

Figure 10. Conidia of *P. palmarum*. A-C. B5 isolate (scales bar = 10 µm)

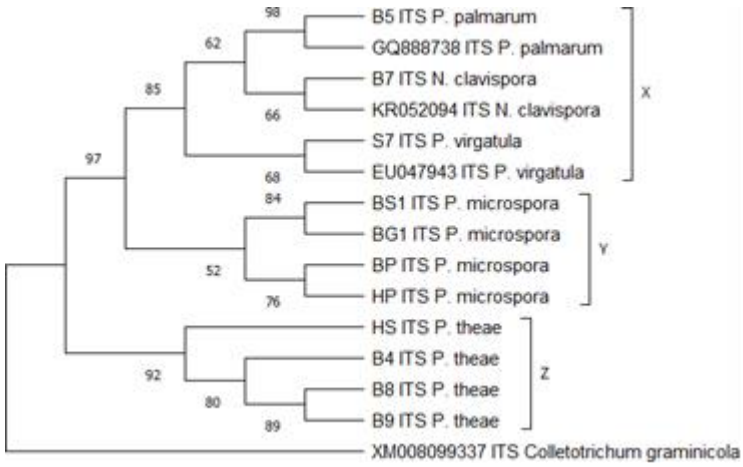

Figure 11. Phylogeny tree of *Pestalotiopsis* isolates based on the ITS gene produced using the maximum likelihood algorithm

Phylogeny analysis

The phylogeny was constructed into 3 clades based on the 11 samples observed and 3 samples from GenBank. Clade X included B5, B7, and S7 isolates identified as *P. palmarum*, *N. clavispora*, and *P. virgatula*, Clade Y had BS1, BG1, HP5, and BP5 isolates identified as *P. microspore*, and Clade Z included B4, B8, B9, and HS4 isolates identified as *Ps. theae*, as presented in Figure 11.

Discussion

Plant disease indicate deviations from the physiological processes of plants and those are attacked usually show certain symptoms. The symptoms on jabon leaves included leaf spot and leaf blight, which leads changes in the color of leaves. A total of 11 isolates were analyzed with 5 linked to red jabon (*Neolamarckia macrophyllus*) retrieved from Kendari, while the remaining 6 associated white jabon (*Neolamarckia cadamba*) consist of 3 from Sukabumi, 2 from Pandeglang, and 1 from Bogor. It was discovered that the symptoms indicated by the changes in the leaf color as well as the damage to the leaf tissue was caused by *Pestalotiopsis*.

Leaf spot caused by *Pestalotiopsis* species appeared as brown spot, which later became large, irregular, and cover the entire leaf (Jeon et al. 2007). It is important to note that *Pestalotiopsis* has a range of hosts (Jeewon et al. 2004) and also causes several diseases, such as cancer, tip death, and fruit rot (Espinoza et al. 2008) in addition to leaf spot and leaf blight. It is a weak pathogen which causes little damage to plants, but some species can lead to serious damage (Hopkins and McQuilken 2000). Moreover, *Pestalotiopsis* is not only exist as pathogens in plants with some living as saprobes in soil or plants and endophytes in plants (Wei et al. 2007). This was confirmed by Maharachchikumbura et al. (2011) that several species are living as endophytes but become dormant when plants are under stress due to environmental activities, pests, or viruses. The colony of *Pestalotiopsis* mycelium was observed as white to brown color and acervuli were noticed on several isolates on day 14 of incubation. The fungus normally produces acervuli on 14th day of incubation, but some species produced faster, while some are slower (Starosta 2004). This means that acervuli may appear radially, spread irregularly, or be placed at the middle. Moreover, the growth of mycelium can be influenced by temperature (Espinoza et al. 2008) and the host (Maharachchikumbura et al. 2011). The morphology of *Pestalotiopsis* colonies also varies significantly even under similar growth conditions, such as changes in the color, texture, and growth rate in the first, second, and third cultures (Hu et al. 2007). Solarte et al. (2017) reported that there is no correlation between mycelium growth and colony morphology *Pestalotiopsis* species were differentiated based on the morphology of conidia (Inanova 2016; Watanabe et al. 2010) which consisted of 5 cells i.e., one apical cell, 3 median cells, one basal cell, and 4 septa.

The conidia have fusiform, straight, or curved cell edges and additional sections at both ends such as flagellum (Hertz 2016). Some of the attributes normally used to distinguish *Pestalotiopsis* species are conidia length and width, number of apical appendages, and length of apical appendage (Wei and Xu 2004). It has been previously reported that size of *Pestalotiopsis* conidia is constant in different media and environments, but the length of the apical appendage and the basal appendage tends to vary (Hu et al. 2007) and also observed for the number and shape of apical appendages as well as the presence or absence of branches (Maharachchikumbura et al. 2014). Moreover, *Pestalotiopsis* can also be grouped based on the median cell color which includes the concolorous and versicolorous pigments (Jeewon et al. 2003).

Pestalotiopsis species isolated from different hosts have diverse morphology and need to be grouped according to their hosts and species (Arvindganth et al. 2016). It has been reported that more than 200 species have similar morphology (Liu et al. 2007), making it difficult to identify the species in addition to the limited morphological characteristics in hosts and different environments (Maharachchikumbura et al. 2016). This means that morphological data needs to be integrated into molecular data to avoid mistakes in the identification process (Ayoubi and Soleimani 2016). The results of identification conducted using BLAST-N showed that there were 5 species of *Pestalotiopsis* from 11 isolates and these include *Ps. theae, N. clavispora, P. palmarum, P. virgatula, and P. microspora*. The *Pseudopestalotiopsis theae* was found in Kendari and Sukabumi, *Pestalotiopsis microspora* in Sukabumi, Bogor and Pandeglang, *Pestalotiopsis virgatula* in Sukabumi area, while *Pestalotiopsis palmarum* and *Neopestalotiopsis clavispora* were found in Kendari. The fungus was also divided into 3 genera which include *Pestalotiopsis*, *Pseudopestalotiopsis*, and *Neopestalotiopsis*. The *Neopestalotiopsis* have versicolorous median cells, while *Pestalotiopsis* and *Pseudopestalotiopsis* are concolorous, but can be distinguished based on their conidiophores and sequence data (Hertz 2016). Referring to the literature (Maharachchikumbura et al. 2011), the results of this study have been compared as follows: The characteristics of the species are different with *Ps. theae* and *P. microspore* observed to have concolorous median cells but *Ps. theae* has knobbed apical appendage and *P. microspora* has a tubular apical appendage. The other species including *P. palmarum, N. clavispora*, and *P. virgatula* were discovered to have versicolorous median cells but *P. virgatula* has a smaller conidia size of 18.1-25.4 x 5.9-6.9 µm than *P. palmarum* with 20.4-27.4 x 5.1-6.8 µm and *N. clavispora* with 22.0-30.5 x 5.1-6.7 um. Meanwhile, *N. clavispora* has a larger conidia size than *P. palmarum* and *P. virgatula*. The findings also showed that *P. palmarum* has a shorter basal appendage with 6.1-10.8 µm compared to *N. clavispora* with 22.0-30.5 µm and *P. virgatula* with 13.9-17.7 µm.

According to Liu et al. (2010), pigmentation in *Pestalotiopsis* is influenced by the environment and the host and is the reason for the variation in median cell color of some isolates. Guo et al. (2016) also reported that *P. microspore* species has versicolorous median cells with the upper two are darker color than the lower one. Jeon et al. (2007) observed that the middle median cell has a darker color than the upper and lower ones.

All the 5 species have been identified as pathogens in several plants, including *Pseudopestalotiopsis theae* which has been reported to cause leaf blight disease in tea leaves (Joshi et al. 2009), in the leaves of *Pinus merkusii* seedlings (Sutarman et al. 2004), and also from the seeds of *Diospyros crassiflora* (Meli and Langer 2009). *P. microspora* causes several diseases, such as kiwi fruit rot (Li et al. 2016) and leaf spot on rubber (Ngobisa et al. 2018), while *Pestalotiopsis palmarum* attacks plants in the palm families, such as the Mexican palm (Douira et al. 2014), *Roystonea elata* (Valentina et al. 2014), as well as others including coconut, sago, banana, and woody plants such as rubber (Abad et al. 2008). *Neopestalotiopsis clavispora* was detected as a pathogen causing cancer and twig dieback in blueberry (Borrero et al. 2018) as well as stem rot in avocado (Valencia et al. 2011). *Pestalotiopsis virgatula* was reported to cause leaf spot disease in eucalyptus (Suwannarach et al. 2012) and fruit rot in rambutan (Keith and Matsumoto 2013). Furthermore, *Ps. theae, N. clavispora*, and *P. microspora* were also found as endophytes in *Podocarpaceae* and *Theaceae* (Maharachchikumbura et al. 2011; Wei et al. 2007), while *P. virgatula* was endophytic in mangrove species (Ronsberg et al. 2013; Liu et al. 2012).

The phylogeny tree divided the 11 isolates of *Pestalotiopsis* into 3 clades based on their morphological characteristics, such as color of the median cell and shape of the apical appendage (Maharachchikumbura et al. 2012). There were 5 species of *Pestalotiopsis* that have the ability to cause leaf spot and leaf blight in jabon, these include *Ps. theae* in B4, B8, B9, and HS4 isolates, *P. microspora* in BS1, BG1, BP5, and HP5 isolates, *P. palmarum* in B5 isolate, *N. clavispora* in B7 isolate, and *P. virgatula* in S7 isolate. All the isolates were differentiated by conidial size, median cell color, as well as the shape and size of apical appendage. Moreover, phylogeny analysis classified all the species into 3 clades based on their median cell color and apical appendage shape. Clade X consisted of *P. palmarum, N. clavispora*, and *P. virgatula* with versicolorous median cell, clade Y consisted of *P. microspora* with concolorous median cell and tubular apical appendage, while clade Z included *Ps. theae* with concolorous median cell and knobbed apical appendage.

The findings showed that *Pestalotiopsis* is a pathogenic fungus with a wide range of hosts and the potential to cause economic losses. This is also an endophyte, and this means that its use needs to be developed further in the future.

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