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## Fungal diversity inhabiting tissues of ebony (*Diospyros celebica* Bakh.) in urban forest

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## Fungal diversity inhabiting tissues of ebony (*Diospyros celebica* Bakh.) in urban forest

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**Abstract.** Ebony (*Diospyros celebica* Bakh.) is an endemic tree species of Sulawesi Island including Central Sulawesi, West Sulawesi and South Sulawesi. This species is also called fancy wood; its color is black-striped reddish brown, beautiful, and luxurious. In addition, tree growth is influenced by microbes, including fungi. Fungi are heterotrophic and eukaryotic organisms absorbing organic compounds from other organisms. The study aimed to identify ebony-associated fungi in Urban Forestry at the Tamalanrea Campus, Hasanuddin University, Makassar, Indonesia. This study consisted of the isolation stage both direct and dilution methods, the rejuvenation stage, and fungal identification. The study result indicated that there were 60 fungal isolates isolated from the ebony tree tissues, while 19 fungal isolates were isolated on the soil under the ebony stands. The direct isolation-based method was higher in term of number of fungal isolates than the dilution-based method. The isolated fungi belonged to the seven genera, namely *Aspergillus*, *Penicillium*, *Gliocladium*, *Trichoderma*, *Fusarium*, *Rhizopus*, and *Phytophthora*. *Aspergillus* and *Penicillium* was genera dominated both in tree tissues and in the soil under ebony stands.

### 1. Introduction

Ebony (*Diospyros celebica* Bakh.) is one of the leading tree species in Indonesia which has higher economic value than other tree species [1–3]. Ebony is an endemic tree naturally growing in Sulawesi Island including Central Sulawesi, West Sulawesi, and South Sulawesi [4]. It is also called as black wood which has various local names such as *Kayu Lotong* (Buginese) and *mouton* (Kaili language), while in the global trade name it is also named as Indonesian ebony, Macassar ebony, Coromandel ebony, straked ebony, black ebony, Sulawesi ebony [5].

Ebony grows on various soil types e.g., limestone, sandy, clay, rocky, and dry soil as well. The ebony wood is unique, attractive, and high quality making its market price is higher. The ebony wood is the first class for strong and durable traits [6]. In addition, it is also called luxury wood which is reddish brown-striped black, beautiful, attractive, and hardwood and has decorative value [7]. In fact, the previous survey-based study to natural-distributed sites showed that ebony's population declined and was difficult to find out the stands.



Both National (PP No 7 of 1999) and international (IUCN) scales, ebony was classified into an endangered tree species and included in a vulnerable conservation status (VU, A1c) which is experiencing a high risk of extinction in fast due to destructed and overexploitation habitat [8,9]. The ebony tree growth is affected by microbes or microorganism or small-sized organisms including fungi.

Microbes can inhabit in variety of habitats since microbes are able to adapt to all kinds of environmental conditions [10]. Microorganisms can perform to interact with each other providing various effects, both beneficial and negative sides [11]. In earlier study, soil fungi living in the rhizosphere area are able to increase the availability of phosphate taken easily up by plants [12]. While the harmful fungi usually live as parasites on the roots, stems, leaves, and fruits.

Study of the identification of fungi on plants has been carried out including on ebony, but it was not carried out thoroughly and only focused on the identification of fungi on the roots of plants. Therefore, this study was carried out to identify the diversity of fungi associated with ebony's tissues including the leaves, bark, stems, and roots, as well as the surface soil under the ebony tree.

## 2. Materials and methods

The study was performed from September to December 2020 in the urban Forest of Tamalanrea campus, Hasanuddin University, Makassar, Indonesia. The fungal identification was carried out in the Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Hasanuddin University.

The tools used in the study were writing stationery, camera, scissors, knife, analytical balance, oven, beaker, measuring cup, test tube, hot plate magnetic stirrer, Erlenmeyer, Laminar Air Flow (LAF), autoclave, petri dish, needle preparation, cork borer, Bunsen, lighter, test tube, micropipette, vortex, glass slide, cover glass and microscope. While the materials used in this study were Potato Dextrose Agar, aquadest, glucosa, labels, plastic wrap, alcohol 70%, and aluminium foil.

### 2.1. Sampling

The samples used were six samples of leaves, barks, stems, roots, and top soil, respectively. Sampling was carried out not only based on location, but also based on tree height and diameter. Leave samples harvested using scissors as many as 3 pieces, then put in a plastic bag. 5 gr barks, 5 gr roots, 5 gr stems, and 5 gr free-litter were taken using a sterile knife and put in the plastic bags followed by labeling them. All tools used in sampling must be sterilized using 70% alcohol. All samples were put into the coolbox for avoiding contamination.

### 2.2. Making media and solution

Media PDA 19.5 g was added with 10.5 g agar, 5 g glucose, and 0.5 L distilled water into the aluminum foil-covered erlenmeyer. The solution was heated and stirred for getting homogeneous solution. Erlenmeyer was covered with aluminum foil then heated. The solution was heated on a hot plate and stirred using a magnetic stirrer until homogeneous solution. The solution was sterilized for 15 min at 121°C followed by pouring the solution into the cup and then covered and glued using plastic wrap to avoid contamination.

### 2.3. Fungal isolation

The PDA media was heated using Bunsen followed by placing samples on PDA media, then covered and glued using plastic wrap. Fungal colonies grew well in the fourth day. Samples of leaves, barks, stems and roots were cut into small pieces and were smoothly grind. 1 g of each samples was coated with aluminium foil and then put into new tube containing 9 ml distilled water and homogenized for 5 min in the vortex.

The first dilution with ratio of 1:10 or  $10^{-1}$ , 1 ml sample was taken using a micropipette into a new tube. The second dilution with ratio of 1:100 or  $10^{-2}$ , 1 ml sample was taken using a micropipette into new tube. Take 1 ml of the solution from the first dilution (tube 2) and then put in into tube 3 to get dilution 2 with ratio 1:100 or  $10^{-2}$ .

1 ml was put into the second dilution (tube 3) and then put into tube 4 to get dilution 3 with a ratio 1:1000 or  $10^{-3}$  and each dilution is vortexed. The solution was taken using a micropipette from dilution of  $10^{-2}$  and  $10^{-3}$  as much as 0.5 ml and was dropped into the surface of the PDA media using a spatula, then covered and glued using plastic wrap to prevent contamination. Fungal colonies appeared for  $\pm 4$  days after isolation.

#### 2.4. *Rejuvenation and purification*

1 fungal colony was taken for purifying perform using a sterilized cork borer and a sterilized needle. Put the fungus was put into the PDA media, then the lips, the petri dish was heated was then closed and glued using plastic wrap to prevent contamination. Observe the fungal growth was observed for about 7 days.

#### 2.5. *Fungal identification*

The fungus was taken using a needle preparation, then placed on a glass slide using the point method, then dripped with distilled water, after which it was covered with a cover glass. The fungus was observed using a microscope to identify its morphological characteristic. Based on book of the Pictorial Atlas of Soil and Seed Fungi [13]. Identification of the fungus was carried out descriptively, determining the genus level by looking at macroscopic characteristics, including color, diameter and texture, microscopic characteristics including hyphae and spores.

#### 2.6. *Observation variable and data analysis*

The variables used in this study refer to the law of fungal identification, namely pictorial, by looking at the morphological characteristics of the fungus such as shape, color, hyphae, spores and mycelium. The results of the study were carried out by descriptive analysis with qualitative methods, namely by looking at the type of fungus that grew on the sample, then looking at morphological characteristics such as color shape, hyphae, spores and mycelium.

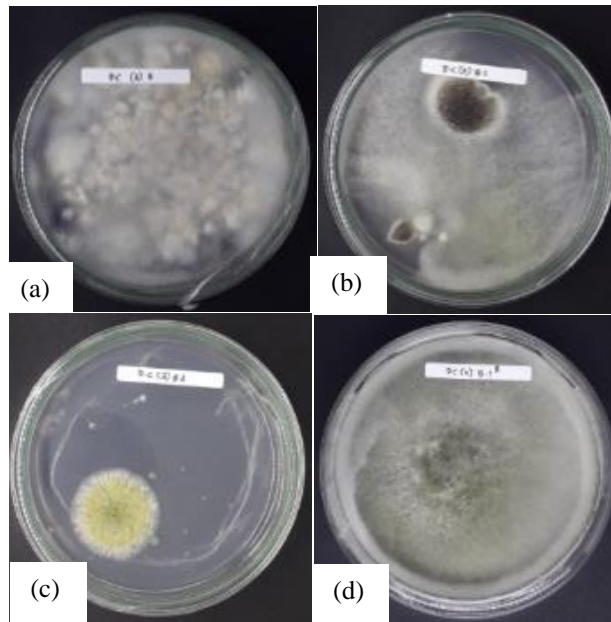
### 3. **Result and discussion**

#### 3.1. *Host tree description*

The number of sampled ebony trees was six trees with distinguished locations. Tree 1 had 13 cm in diameter and 5.9 m in height collected from Unhas postgraduate school area. Tree 2 had 6.3 cm in diameter and 3.9 m in height collected from Unhas postgraduate school area. Tree 3 had 38.5 cm in diameter and 21.1 m in height collected from the Unhas Institute for Research and Community Service area. Tree 4 had 9.8 cm in diameter and 5.5 m in height collected from the Unhas mosque area. Tree 5 had 7.6 cm in diameter and 6.1 m in height collected from the Unhas mosque area. Tree 6 had 7.9 cm in diameter and 4.7 m in height collected from the faculty of pharmacy area.

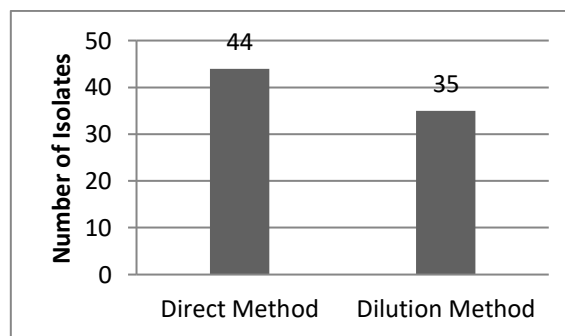
#### 3.2. *Fungal Isolation*

The fungal isolation process allowed fungus to grow and form a colony that overlaps with other colonies. Since then, a purification stage was carried out to obtain pure fungi based on their morphological appearance. Figure 1 (a) which is a fungal isolate isolated from the root of tree 2 using direct method, showed that there were many fungal colonies growing but having the same morphological appearance. Figure 1 (b) is a fungal isolate isolated from a root of tree with 2 (dilution  $10^{-2}$ ) showing that there are 3 different growing fungi with different morphological appearances based on their color. Figure 1 (c) is a fungal isolate isolated from a root of tree 2 (dilution  $10^{-3}$ ), indicating that there was 1 fungal colony and bacteria were appeared. While Figure 1 (d), which is an isolate from a root of tree 2 (dilution  $10^{-2}$ ), was successfully purified and did not overlap with other isolates.

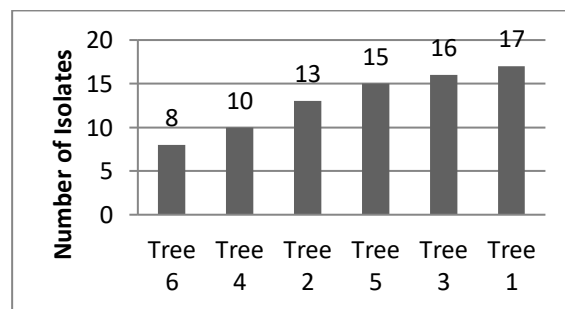


**Figure 1.** Fungal isolation (a) Direct method, (b) Dilution method  $10^{-2}$ , (c) Dilution method  $10^{-3}$  and (d) The result of purified isolate

The number of fungal isolates that were successfully isolated inhabiting both ebony tissues and soil was 79 isolates. Based on the isolation method with the dilution method  $10^{-2}$  had the highest number of isolates compared to the dilution method  $10^{-3}$ .



(a)



(b)

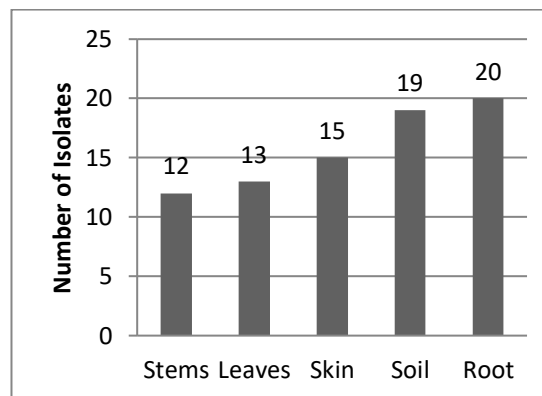
**Figure 2.** Number of Isolates based on (a) Direct and dilution methods and (b) Six tree samples

The result of isolation and purification of fungi from 6 ebony trees had various isolates and types of fungi. The difference in the number of isolates obtained is due to different environmental factors from one tree to another (Figure 2).

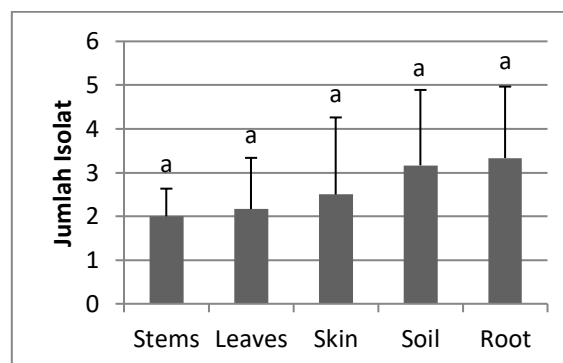
**Table 1.** Correlation analysis of tree diameter and height on the number of isolates

		Diameter	Height	Number of Isolates
Diameter	Coef	1	0.9888	0.4573
	p-value		0.0002	0.3618
	N	6	6	6
Height	Coef	0.9888	1	0.4424
	p-value	0.0002		0.3797
	N	6	6	6
Number of Isolates	Coef	0.4573	0.4424	1
	p-value	0.3618	0.3797	
	N	6	6	6

The number of isolates found in various ebony tissues and the soil below the surface of the ebony tree had different amounts. The highest number of isolates was found in the roots and stems and the lowest number of isolates was found in the stems (Table 1).



(a)



(b)

**Figure 3.** (a) Number of fungal isolates based on tree tissues (Host) and (b) The average number of fungal isolates and its standard error

The fungal diversity both in trees and soil can be known by the isolation stage. The isolation was performed by taking microbes from their natural environment, followed by growing them as pure cultures in artificial media [14]. The isolation method of microorganisms can be done by spreading, scratching and sowing. The spread method is an isolation technique by inoculation of microbial cultures on the surface of PDA.

Two methods were used, namely the direct method and the dilution method ( $10^{-2}$  and  $10^{-3}$ ). Microbes growing during the isolation process formed colonies that overlap with other colonies (Figure 1 a, b, c). Therefore, a purification step was carried out to separate each colony according to its macroscopic morphological appearance so that it would get a completely pure or uncontaminated colony (Figure 1 d). Isolation activities in this study, especially in the group of fungi whose colony growth was seen in 2-5 days after isolation.

Figure 3 shows that the direct method had the highest number of isolates compared to the dilution method ( $10^{-2}$  and  $10^{-3}$ ), namely 44 isolates and 35 for the dilution method. The number of isolates in dilution  $10^{-2}$  was more than in dilution  $10^{-3}$ , namely 25 isolates for dilution  $10^{-2}$  and 10 isolates for dilution  $10^{-3}$ . The direct method did not separate/reduce the number of microbes so that more microbes were isolated. The dilution method is usually done in decimal  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and so on. Dilution is carried out to separate the microbial cells that have merged into one. Dilution aims to minimize or reduce the number of microbes suspended in the liquid.

The obtained 79 isolates and each tree differed in the number and types of fungi obtained. Figure 4 shows that the highest number of fungal isolates is in tree 1 with a total of 17 isolates, while tree 6 with the lowest number of isolates is 8 isolates. The result of microbial isolation showed that the number of isolates obtained was different in each location. The difference in the number of isolates obtained was due to the different environments between tree 1 and other trees. Nasution et al., (2017) said that environmental factors such as soil pH, humidity, temperature, light intensity and altitude are one of the factors that influence the development and growth of fungi [15]. Apart from these factors, it also depends on the type of fungus because each type of fungus has a different vulnerability to life in a habitat.

Microbes can be found everywhere, for example, in water, soil, air, plants, animals and humans. The surface of plant that is in contact with the air contains various kinds of microbes, including the potential as a plant nuisance (pathogen) as well as beneficial microbes. According to Meyer and Leveau (2012) divide microbes into two, namely deficit microbes found in the inner tissues of plants and phyllospheric microbes, which are airborne microbial colonies found on plant surface (leaves, bark, stems, etc.) [16]. Fungi can grow on weathered wood, soil and litter. However, there are several types of fungi that grow on living stems that are attached to the outer layer of the stem [17].

Based on the correlation test in table 1 of three parameters, namely, tree diameter, tree height and a number of isolates, it shows that tree height has a high correlation value compared to the correlation value of tree diameter on the number of isolates. Tree height has a low correlation to the number of isolates which shows the number 0.3797. While the diameter of the tree also has a low correlation with the 0.3618. The relationship between tree diameter and the number of fungal species has a positive relationship [18].

The fungus grows more on the roots and soil when compared to the leaves, bark, and trunks of ebony trees (Figure 5 (a)). The leaves and stems of the ebony tree had almost the same number of fungal colonies, namely on the ebony stems with a total of 12 colonies, and the leaves, there were 13 colonies of fungi. The presences of fungi vary greatly in each plant, both the same and different species. Fungi are able to colonize every organ of the plant, one of which is the leaves.

Several studies have shown that leaf age can affect fungal density of leaves. Old leaves support more fungus than young leaves. Meanwhile, the leaf samples taken were semi-old leaves (between young and old leaves). The study of Hilarino et al (2011) found fungi with a higher frequency of colonization on old leaves than on young leaves [19].

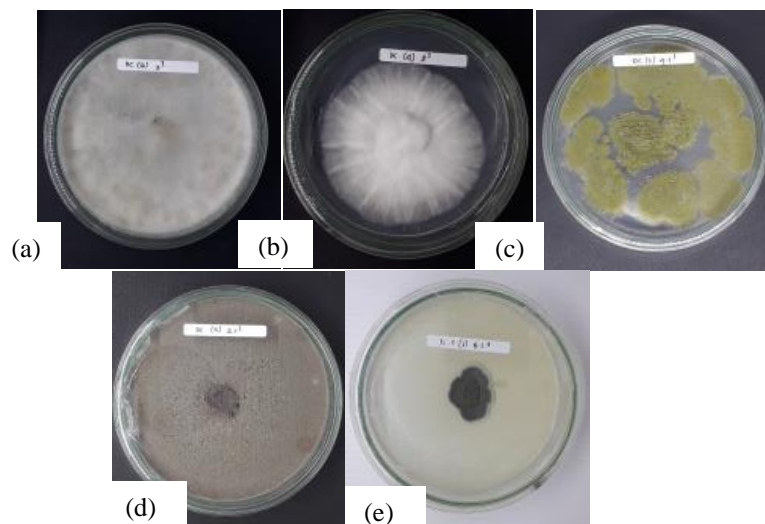
The rhizosphere environment is rich in energy sources from organic compounds released by plant roots (root exudates) and habitat for various types of microbes to thrive [20–22]. Root exudates determine the diversity and number of microorganism populations. According to Simatupang (2008),

the population of microorganisms in the rhizosphere area is more numerous and varied than in non-rhizosphere soil [23].

The activity of rhizosphere microorganisms is influenced by the exudate produced by plant roots. Meanwhile, according to Roberts et al., (2016), microorganisms are most often found in the upper soil layer, for example, the soil surface layer, because this layer is most likely to be exposed to fertilizers and in direct contact with organic intakes such as leaves [24]. Infertile soil, there are more than 100 million microorganisms/gr. The complexity of the nutrients for the growth of fungi in the soil causes the fungus that grows to be very diverse [25]. Figure 5 (b) shows that the comparison is not significant (not significantly different) between the number of isolates found in various tissues with a value of  $F_{\text{count}} < F_{\text{table}} 5\%$ , which is  $0.46 < 2.6$ . This means that the ebony tree tissue does not determine the number of fungal isolates caused by microbes that can grow in various habitats. However, physical environmental factors are still very supportive of the growth and development of fungi. Swasono (2018) said that mushrooms need a host that provides carbohydrates, proteins, vitamins and other chemical compounds to meet their needs [26].

### 3.3. Morphological characteristics and identification of fungi

The morphological characteristics of 79 isolates of fungi growing on PDA media included 17 isolates on tree 1, 13 isolates on tree 2, 16 isolates on tree 3, 10 isolates on tree 4, 15 isolates on tree 5 and 8 isolates on tree 6. The fungi found were widely varied based on their morphological appearance in term of isolate color, isolate diameter and texture. The identification stage was carried out using a microscope after the fungus had grown for seven days on PDA media. The identification of the fungal isolate was carried out microscopically by observing the vegetative structure (hyphae) and generative structure (spores). The results of fungal observations were compared with the fungal determination key book Pictorial Atlas of Soil and Seed Fungi (Watanabe, 2010) so that we can find out the genus of the observed fungus [13]. The study result showed that each fungal colony had variety colors, that their color is one of the variables to identify fungal isolate.



**Figure 4.** Fungal Colony Color (a) White Slightly Brown, (b) White, (c) Green, (d) Gray, and (e) Black

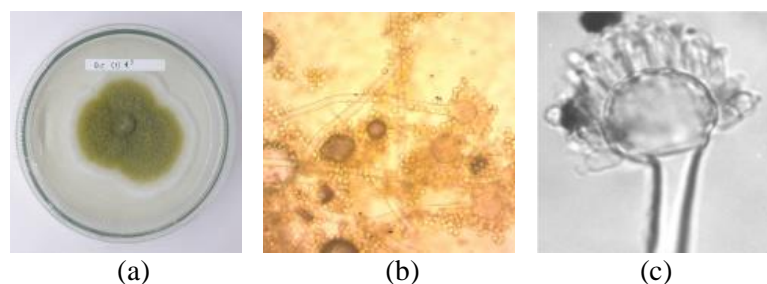
Figure 4 shows the colors of the fungal colonies on the ebony tree tissue and the soil beneath the ebony tree surface. The morphological characteristics of fungi growing on PDA media include 17 isolates of fungus on tree 1, 13 isolates on tree 2, 16 isolates on tree 3, 10 isolates on tree 4, 15 isolates on tree 5, and 8 isolates on tree 8. The ebony tree tissues and the soil around the surface of the ebony

tree are very diverse (see Table 2). This diversity is in the form of the color of the fungal colonies on the top and bottom have different colors, as well as various fungal textures with different hyphae growth rates for each isolate. Colony diameter ranged from 1,2 – 8,3 cm, 10 cm (full) to spread in the form of spots. The colony texture was dominated by fine cotton but some isolates had a texture in the form of coarse cotton and velvet. Fungal colonies also had various colors (Figure 6).

Table 2 shows that there are 79 isolates of fungi with different genera. The isolates of fungi that were identified were 55 isolates, including *Aspergillus*, *Penicillium*, *Gliocladium*, *Trichoderma*, *Rhizopus*, *Fusarium*, and *Phytophthora*. However, there were 22 isolates that could not be identified correctly which were referred to as fungi X. In the study, fungi from tree 1 were more diverse with 17 species of fungi from five different genera, namely *Aspergillus*, *Penicillium*, *Gliocladium*, *Trichoderma* and *Rhizopus*.

In tree 2 there were 13 colonies of fungi from four genera namely *Aspergillus*, *Penicillium*, *Gliocladium* and *Trichoderma*. Tree 3 consisted of 16 colonies from four genera, namely *Aspergillus*, *Gliocladium*, *Fusarium* dan *Rhizopus*. Tree 4 consists of 10 species of fungi from three genera, namely *Aspergillus*, *Penicillium* dan *Rhizopus*. On tree 5 with species of fungi consisting of four genera, namely *Aspergillus*, *Penicillium*, *Trichoderma*, and *Phytophthora*. While tree 6 with the lowest number of fungal isolates with a total of 8 species of fungi from four genera, namely *Aspergillus*, *Gliocladium*, *Trichoderma*, and *Rhizopus*.

**3.3.1. *Aspergillus*.** The colonies appeared as white filament then changed color depending on the species. The observations showed that the macroscopic characteristics of the *Aspergillus* fungus were black and green isolates with a fine cotton texture. While the microscopic characteristics are conidia spherical with septate and hyaline hyphae. Microscopic observations according to the literature images.



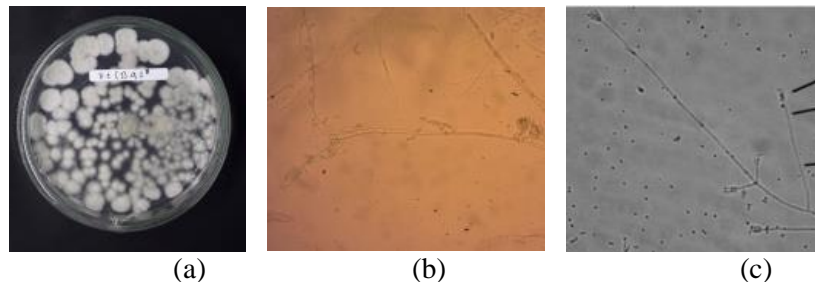
**Figure 5.** (a) Macroscopic-based Observation, (b) Microscopic-based Observation, and (c) Watanabe (1993)-based Literature [13]

At the beginning of the observation, colonies appear as white filaments then change color depending on the species. Observations showed that the macroscopic characteristics of the *Aspergillus* fungus were black and green isolates with a fine cottony texture. This is in accordance with the research of Ristiari et al., (2018) which said that the macroscopic characteristics of the *Aspergillus* fungus on PDA media had a surface that was light green to dark green and black, and a texture like flour [27]. While the microscopic characteristics are conidia spherical with septate and hyaline hyphae.

The observation results (Figure 5) are also supported by the result of Tunggal (2019) that this isolate has black conidiophores stalks and a dark brown round shape [28]. Putra et al., (2020) said that *Aspergillus* is an antagonist fungus that is in the soil and acts as a decomposer [29]. *Aspergillus* is a type of ubiquitous soil mold, especially in tropical and subtropical areas with warm climates. The fungus *Aspergillus* is the soil [30]. Meanwhile, Kelo (2017) showed that the identification of rhizosphere fungi in the ebony community forest of Barru Regency only found fungi of the genus *Aspergillus* [31].

**3.3.2. *Penicillium*.** This isolate appeared as white colonies and grew spreadly and had a cotton and velvet texture. While the microscopic characteristics of the head of hyphae that carry spores are shaped like a broom. In addition, it appeared with white colonies and grows spreadly and has a cotton and velvet

texture, this is in accordance with research by Ristiari et al., (2018) that the surface of the colony is white [27].

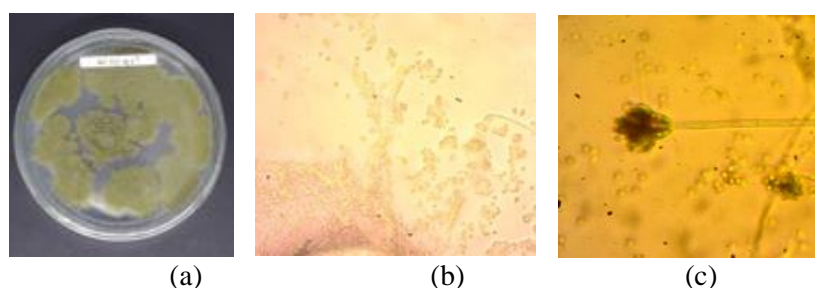


**Figure 6.** (a) Macroscopic Observation (b) Microscopic Observation (c) Purwatisari & Hastuti,(2009)-based Literature [32]

Putra & Purwantisari (2018) specific characteristics of the fungus *Penicillium* sp. are insulated hyphae, branched mycelium, usually colorless, conidiophores insulated and appear above the surface derived from hyphae below the surface of branched or unbranched hyphae, the heads of hyphae carrying spores are shapes like brooms, with sterigmata appearing in clusters, conidia chain-shaped because it appears one by one from the sterigmata (Figure 6) [33]. Species *Penicillium* sp. is a soil fungus that prefers a cool climate and is present when organic matter is available [34].

*Penicillium* sp. protects plants from pathogen attack while increasing plant growth, as well as a decomposer that can increase soil fertility [27]. The study of Saragih et al., (2015) said that *Penicillium* plays a role in dissolving phosphate [35]. The dominant phosphate solubilizing fungi in the soil are *Aspergillus* and *Penicillium* because Indonesia's soil is acidic. The dominant type of *Penicillium* was found in young mangrove leaf tissues. *Penicillium* has a very important function in the growth of an organism that is the host.

3.3.3. *Gliocladium*. Macroscopic observations showed that this isolate was brownish white to green with a cotton-like texture. Microscopically, the conidiophores are in the form of a solid brush, but some are simple centers.



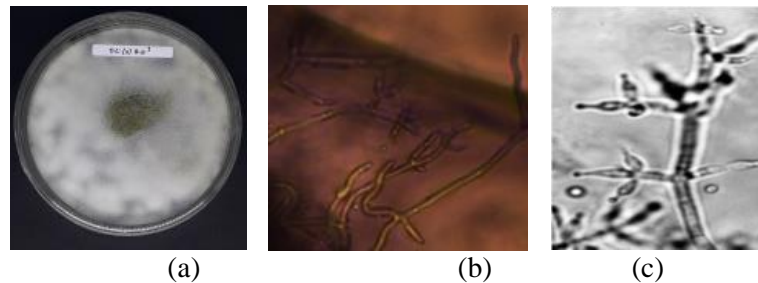
**Figure 7.** (a) Macroscopic Observation, (b) Microscopic Observation, (c) Watun (2018)-based Literature [36]

Figure 7 showed that this isolate was brownish white to green with a cotton-like texture, this is in accordance with Sari (2017), which said that this isolate grew rapidly, the texture was smooth; initially white turned pale to dark green with sporulation [34]. *Gliocladium* sp. similar to *Penicillium* sp. The difference is that the branching supports the mass of spores such as bound or conidia in one head [37].

The genus of the fungus *Gliocladium* sp. conidiophores have straight conidiophores and branching at the ends. The hyphae are compact and in the form of a penicillate structure. Microscopically, the conidiophores are solid brush-shaped, but some are simple centers (Verticillate), single-celled conidia, bright hyaline with smooth walls [38].

*Gliocladium* is a saprophytic fungus that is easily found in various types of soil. These fungi are generally able to suppress pathogens from the soil so that it is beneficial for plants. Soil microorganisms such as *Gliocladium* act as decomposers. In addition, as a biological control agent for plant pathogens, this gives hope to reduce the use of synthetic fertilizers and fungicides [39].

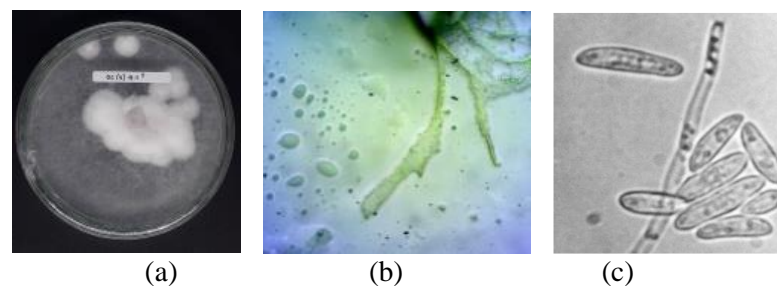
3.3.4. *Trichoderma*. Macroscopically, the colonies were initially white and then greenish. While microscopically, it was shown that *Trichoderma* had branching conidiophores that resembled pyramids.



**Figure 8.** (a) Macroscopic Observation, (b) Microscopic Observation, (c) Watanabe (1993)-based Literature [13]

Gusnawaty et al., (2014) said that *Trichoderma* has branching conidiophores, which resembling a pyramid that is at the bottom of the lateral branches that are repeated, the more the ends of the branches become shorter [40]. Conidia are smooth-walled and semi-spherical to oval in shape. Macroscopically, initially, the colony was white and then the mycelium changed color to yellowish green or dark green, especially in the showed a lot of conidia (Figure 8). Exploration of primary forests in several areas shows that *Trichoderma* is generally found in rooting soils. This *Trichoderma* fungus is a fungus that is commonly found in the soil and can multiply rapidly in the roots. *Trichoderma* has antagonistic properties against pathogenic microbes against plants so that it has the potential to be used as a biocontrol against plant pathogenic microbes [41]. Miranti et al., (2015) research said that *Trichoderma* is commonly found in tropical and subtropical areas [30].

3.3.5. *Fusarium*. The macroscopic-based observation showed that the fungus isolate was white with cottony texture. The microscopic-based appearance showed that the fungal colonies found were ovoid or oval in shape.



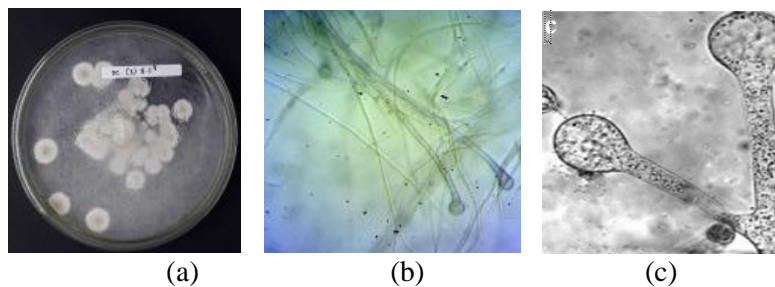
**Figure 9.** (a) Macroscopic-based Observation, (b) Microscopic-based Observation, (c) Watanabe (1993)-based Literature [13]

Macroscopic observations (Figure 9) showed that this fungus isolate was while with a cottony texture, this was in accordance with the observations of Sutejo et al., (2008) that this isolate had white colonies or was accompanied by a purple or pink color at the center of the colony. Isolates that form sporogonium in sufficient quantities, the colony will change color from white to orange [42]. The identification result obtained is in accordance with the results found by Sunarmi (2010), namely the fungal colonies found to be oval or oval in shape, formed singly or in series, and formed a white or pink

mass [43]. In general, *Fusarium* sp., when viewed from a microscopic point of view, it has an ovoid microspore shape, which generally has 0-1 bulkheads, while the macrospore shape is generally long with a pointed tip and has 2-6 bulkheads [44].

*Fusarium* is a genus of fungi that is found in plants and soil. In a study by Sholihah et al., (2019) that there are several *Fusarium* genera that act as decomposers but are also pathogens in a number of agricultural plants [45]. Nugraheni (2010) *Fusarium* is a pathogenic fungus that can plant with a very wide host range [44]. This fungus attacks the vascular tissue resulting in wilting of the host plant.

3.3.6. *Rhizopus*. The macroscopic-based observations showed that the fungus isolate had a gray color with white edges with a velvety or cottony texture. Microscopically, it shows smooth hyphae with sporangiophores that are not too long and sporangium round.

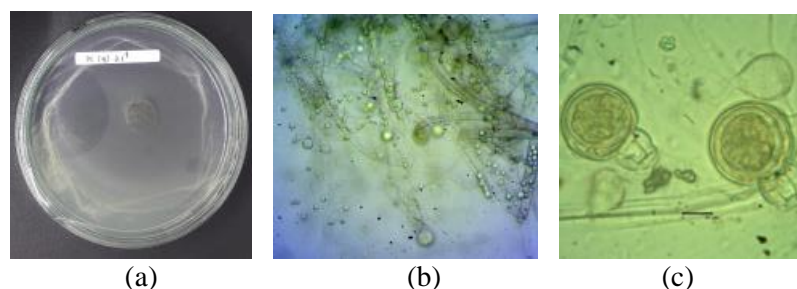


**Figure 10.** (a) Macroscopic Observation, (b) Microscopic Observation, (c) Watanabe, (1993)-based Literature [13]

Macroscopic observations (Figure 10) showed that this fungus isolate had a gray color with white edges with a velvety or cottony texture. Macro identification is in accordance with the research of Virgianti (2015), which states that the characteristics of the colony are grayish white mycelium with blackish gray spores and smooth hyphae with not too long sporangiophores and round sporangium [46]. Microscopic observations were single sporangia and spherical spores [47].

Several types of soil fungi, one of which *Rhizopus*, has a role as PGPF (Plant Growth Promoting Fungi). *Rhizopus* is a type of mold that is easy to grow in any environmental condition but is dominantly found in the soil. *Rhizopus* can also produce compounds that can inhibit pathogenic bacterial and function as antioxidants [48].

3.3.7. *Phytophthora*. The macroscopic-based observation showed that the isolate was black with a cotton texture. The fungus had a mycelium consisting of slender non-septated hyphae.



**Figure 11.** (a) Macroscopic-based Observation, (b) Microscopic-based Observation, (c) Rohmah et al., (2018)-based Literature [49]

Macroscopic observation (Figure 11) showed that the isolate was black with a cotton texture; this was in accordance with Setyowati (2018), which said that macroscopically the color of this fungus colony was white and black in the middle [50]. This fungus has a mycelium consisting of slender non-

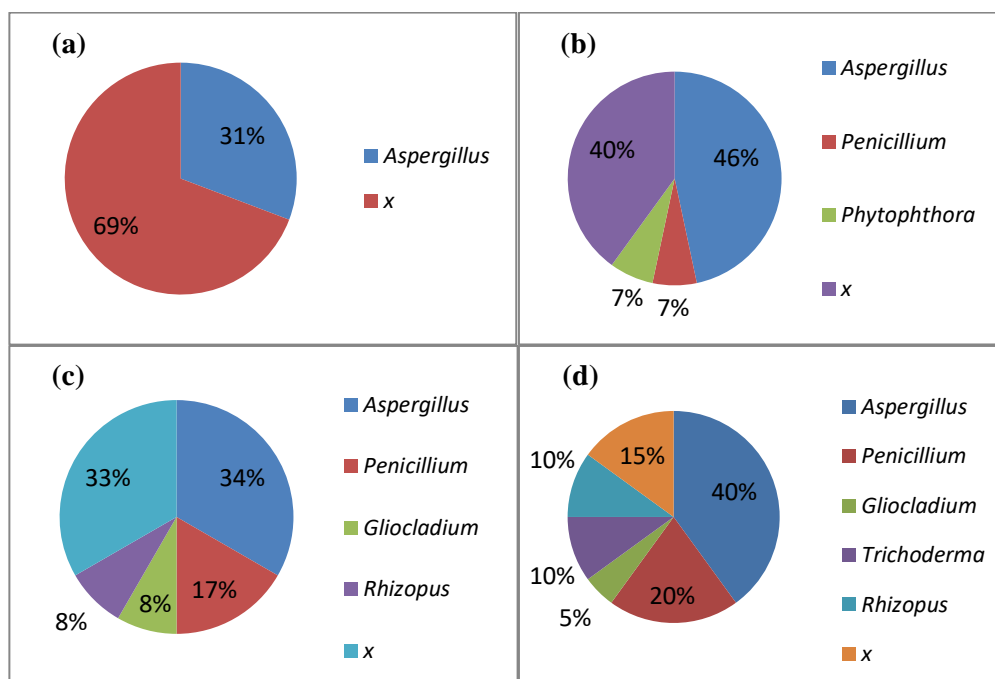
septated hyphae. The sporangia produced are specific in sympodial, the sporangiophores are branched with unlimited growth [34].

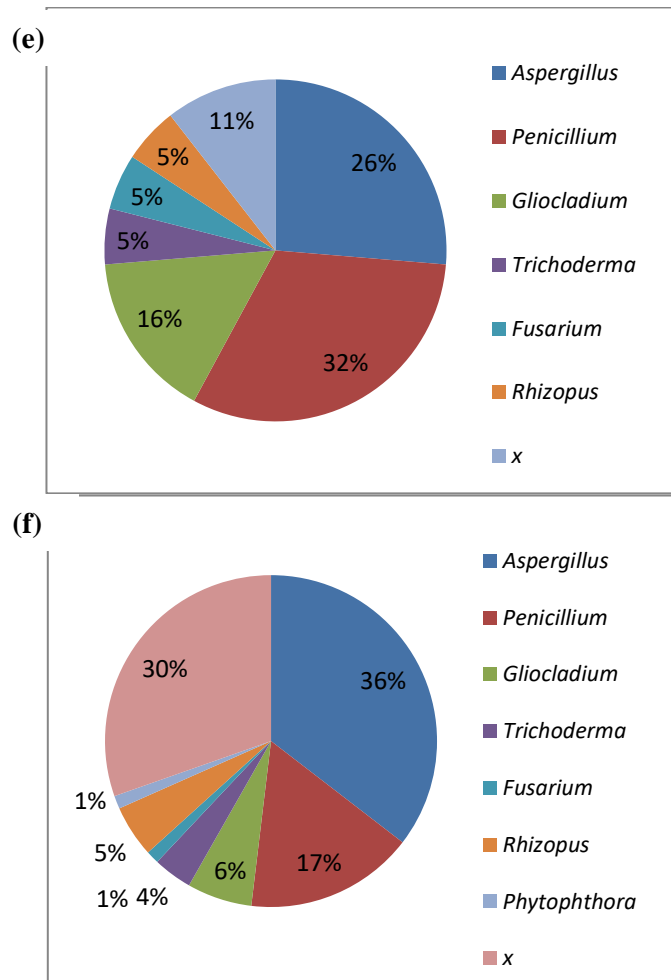
Wahyuno (2009) said that *Phytophthora* is a pathogen that causes fruit fall and rot disease in plants [51]. So, it is known that *Phytophthora* is a fungus that is known as a pathogen in the agricultural world. *Phytophthora* attacks the roots, stems, leaves, and fruits of plants. Figure 14 shows that *Aspergillus* is a fungus that grows on all tree tissues (hosts). This fungus can be found in air, vegetables, soil, and humus [52].

While *Phytophthora* is a fungus that is only found on the bark of the ebony tree. Fungi of this genus usually grow on fruit, bark, and stems of plants, but in general, these fungi are pathogenic. In the study, the dominant genera were found, namely the genus *Aspergillus*, *Penicillium*, and *Gliocladium* (Figure 12).

The results of the study were supported by Kamase (2019) and Watun (2018), saying that the fungi found in the rhizosphere area on teak and candlenut trees were *Aspergillus*, *Penicillium*, *Gliocladium*, *Rhizopus*, and *Fusarium* [36,53]. Herlina (2013) and Rahmi et al. (2017) also said that *Aspergillus*, *Penicillium*, and *Gliocladium* are fungi that are found abundantly in nature, tree residues, and are scattered in various types of soil, such as forest soils and in various plant rhizospheres [39,54].

Meanwhile, Budiarti & Nurhayati (2014) research said that *Trichoderma*, *Penicillium*, and *Aspergillus* are fungi that are commonly found in the soil and grow quickly [55]. *Aspergillus* genus found in all tree tissues and soil beneath the surface of ebony trees, while the *Phytophthora* genus with 1 isolate was only found on the bark of ebony trees.





**Figure 12.** (a) Percentage of Number of Genus in the Leaves, (b) the bark, (c) the Stems, (d) the Roots, (e) the Soils, (f) all Tree tissues and soil

*Aspergillus*, *Penicillium* dan *Gliocladium* were the most common fungal genera found in tree tissue and soil.

#### 4. Conclusion

Based on the results of the research on the identification of fungi on the ebony tree, it was shown that the isolates of the fungus on the ebony tree tissue contained 60 isolates of the fungus, while the soil under the surface of the ebony tree contained 19 isolates of the fungus. The direct isolation method produced more isolates than the dilution method. The correlation between tree height and ebony tree diameter had a positive correlation with the number of fungal isolates, but the correlation was not significant. The fungi found on the ebony tree tissue and on the soil below the ebony tree are included in seven fungal genera, namely *Aspergillus*, *Penicillium*, *Gliocladium*, *Trichoderma*, *Fusarium*, *Rhizopus*, and *Phytophthora*. 22 isolates of fungi that have not been identified.

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