Isolation and identification of decomposer fungi from *Macaranga indica* and *Hibiscus macrophyllus* leaf litter from restoration area of Gunung Leuser National Park

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**Abstract.** Leaves mostly litter on the forest floor. The process of leaf litter decomposition involves the role of microorganisms such as fungi. The study aimed to isolating and identifying the fungi of *Macaranga indica* and *Hibiscus macrophyllus* leaf litter. The leaf litter was taken under the stand of *Macaranga indica* and *Hibiscus macrophyllus* in the restoration area of Gunung Leuser National Park Sei Betung Resort North Sumatra. Fungi were isolated using the direct plating methods with Potato Dextrose Agar medium. The purified fungi were then identified morphologically to the genus level. Morphological identification carried out by looking at the macroscopic and microscopic features of fungi. There were six isolates from *Macaranga indica* leaf litter and eight isolates from *Hibiscus macrophyllus* leaf litter. The result shows that there are five genera of fungi consisting of *Aspergillus*, *Penicillium*, *Mucor*, *Trichoderma*, and *Cladosporium*.

**1. Introduction**

Restoration is an action to restore degraded land like its original condition. Restoration is indispensable if an ecosystem has undergone a change that is very far from its original condition and cannot renew itself naturally to return to its original state. Restoration is also needed if the ecosystem cannot carry out the function properly so that it requires management and protection [1]. Restoration is an effort to restore the biological and non-biological elements of an area to the original species so that the biological balance and ecosystem can be achieved. In the implementation of restoration, it is necessary to consider the selection of the type of plant to be used. According to Arsyad [2], there are several things need to be considered, namely the selection of local plants, the species that are fast-growing, can grow on soil that is nutrient-deficient, and easily multiply and easily cultured. Besides that, easy to plant and maintain and the rate of litter production is high and easily decomposed.

Sei Betung Resort restoration area was previously an oil palm plantation which was later restored to accelerate the formation of forest stands. Restoration efforts were carried out by planting *Hibiscus macrophyllus* (*H. macrophyllus*) and *Macaranga indica* (*M.indica*) trees [3].

*Hibiscus macrophyllus* and *M. indica* are types of plants that can be used as plants for restoration. *Hibiscus macrophyllus* is one of the fast-growing species of the Malvaceae family, and the wood can be used as building material. This tree can grow from a height of 10 m to 700 m above sea level [4]. *Macaranga indica*, including the Euphorbiaceae family, is one of the pioneer plants that can redevelop damaged forests. This plant can be used as a building material and as a traditional medicine [5, 6]. Both types of plants produce quite a lot of litter. Litter that falls will decompose involving various organisms...
including fungi. Fungi are well known as decomposing agents of organic matter. Most of the individuals fungi are saprophytic and are efficient in degradation of major polymers such as cellulose and lignin [7]. Decomposer fungi consist of many species such as Aspergillus, Trichoderma, Eurotium, Penicillium, Rhodotorula, Paecilomyces and Thielaviopsis [8, 9]. Previous studies on the abundance of fungi in leaf litter have been carried out [8–13]. Most studies of fungi on leaf litter are aimed to discovery the new type of fungi. The presence of fungi plays a major role in maintaining the continuity of the nutrient cycle. Therefore, fungi in leaf litter directly play a role in maintaining soil fertility. Studying the role of fungi in decomposition process of plant litter is important because fungi play an important role in controlling the accumulation and release of essential nutrients for plant growth and build up of soil organic matter [14].

The study aimed to isolating and identifying fungi from Hibiscus macrophyllus and Macaranga indica leaf litter.

2. Material and methods

2.1. Study site and sampling procedure
The study was conducted from April to June 2017. Leaf litter was taken at the Sei Betung Resort restoration area of Gunung Leuser National Park. Leaf litter was taken under the stand of Hibiscus macrophyllus and Macaranga indica in a plot of 5m x 5m in five plots. Leaf litter taken at the surface of soil. In each plot, leaf litter was taken 100 g diagonally at five points. The leaf litter was then put into white plastic and stored ice box for transportation from the field to the laboratory to isolating decomposer fungi. Leaf litter placed in chilled room at 4-16 °C prior to analysis.

2.2. Leaf litter analysis
Leaf litter was analyzed for its organic C content by Gravimetric method and N total using the titrimetric method [15]. To get the value of organic C content, ten (10) g leaf litter put in the two cup then put in the oven for 24 hours with temperatures 105 °C dan 70 °C. Determination of C content based of weight loss due to heating. To get the value of N total, 0.25 g of leaf litter that passes the sieve 40 mesh was extracted with H2SO4 and H2O2. Extraction results are then distilled and titrated.

2.3. Isolation of fungi from leaf litter
The isolation of litter decomposer fungi followed direct plating methods [16]. Ten g of each leaf litter was washed with running water, then cut into pieces and placed on a petri dish containing sterile Potato Dextrose Agar (PDA) with calmicetin to restrict bacterial growth, five pieces per petri dish. Petri dish which contains leaf litter peaces then incubated for 5-7 days at 28 °C. The growing fungus colonies were purified by transferring to the new PDA medium, the growing colonies were identified macroscopically and microscopically, then adjusted to the fungi identification book [17, 18]. Fungi are classified up to the genus level. To ensure that the isolated fungi is decomposers, the fungi are grown on Carboxyl Methyl Cellulose (CMC) plus congo red medium. Carboxyl Methyl Cellulose is specific medium for decomposer fungi. Decomposer fungi will form clear zone on CMC medium.

2.4. Calculation of the total number of fungi
The total fungi were calculated using the dilution method [16]. Ten g of crushed leaf litter was put into Erlenmeyer containing 90 ml of sterile physiological (8.5 g NaCl per 1000 ml distilled water) solution and shaken for 15 minutes. The suspension was diluted ten folds. One mL of the 10⁻¹, 10⁻², and 10⁻³ diluted solution was added with ten ml of nutrient agar at 50 °C, then incubated for three days. After incubation, the result is expressed in Colony Forming Unit (CFU) per g of sample.

3. Result

3.1. Analysis of the leaf litter chemical content
One of the factors that influence the decomposition process is the nutrient content of the leaves, especially the carbon content (C) and nitrogen (N) which are related to the carbon-nitrogen ratio. The
higher the carbon and nitrogen ratio, the more difficult the material decomposes. The results of the analysis of carbon and nitrogen content are presented in table 1.

| Type of analysis | Unit | M. indica Leaves | H. macrophyllus Leaves |
|------------------|------|------------------|------------------------|
| Organic C        | %    | 49.49            | 51.11                  |
| Total N          | %    | 2.44             | 2.22                   |
| C/N Ratio        |      | 20.28            | 23.02                  |

The ratio of leaf litter ranges between 20.28 and 23.02. The mineralization process will run faster if the litter content of N is high and the C/N ratio is less than 30 [19]. These results indicate that M. indica and H. macrophyllus leaf litter are rapidly decomposed.

### 3.2. Fungi isolation

All fungi were identified by their cultural and morphological characteristics. Thirteen fungi isolates were found, namely five isolates from *M. indica* leaves and eight isolates from *H. macrophyllus* leaves. They belong to five genera namely *Aspergillus*, *Mucor*, *Penicillium*, *Trichoderma*, and *Cladosporium*.

#### 3.2.1. Aspergillus

*Aspergillus* fungi are found in *M. indica* leaves and on *H. macrophyllus* leaf litter. *Aspergillus* is a fungus that spreads widely both in the waters and tropical regions with colony colors that are generally black, brown, yellow and green [17, 18] (figure 1.). There are eight isolates identified as *Aspergillus*, and there are four types of colonies with different characters. The characteristics of the four colonies are as follows: (1) the colonies grow unevenly, yellowish-green colonies with 3-5 cm in diameter on day seven. Dense conidiophores with conidial heads round to semi-round identified as *Aspergillus* sp.1. There are two isolates identified as *Aspergillus* sp.1; (2) rounded growing colonies at each point on the petri dish with a diameter of 0.5-4 cm after seven days of purification. The colonies are dark brown to black. Conidiophores are thick and spread with conidial heads in around and black shape. This colony is identified as *Aspergillus* sp.2; (3) the colonies grows circular and spreads with 1-7 cm colony diameter, thin black colony color with white base color. The conidiophores are straight in shape with dense spores. Those colonies are identified as *Aspergillus* sp.3. There are two isolates identified as *Aspergillus* sp.2; (4) colonies are dark brown to black, grow round and spread with a diameter of 1.3-5 cm. Conidia are round with a brownish yellow color and thick conidiophores with yellow heads. These colonies are identified as *Aspergillus* sp.4. There were three isolates identified as *Aspergillus* sp.4.

#### 3.2.2. Mucor

*Mucor* is one of the fungi that act as decomposers. The colonies of fungi is yellowish white with a diameter of 5-7 cm. The shape of sporangiophore are semi-round to rounded branch. The colony looks like cotton and is white (figure 2).
3.2.3. *Penicillium*. *Penicillium* is like *Aspergillus* which is a fungus often found to act as a decomposer. These two fungi can grow and develop easily and decompose the substrate they grow. These fungal colonies grow evenly and are greenish with 2-7 cm in diameter. Conidia are round and grow in metula branching (figure 3). Only one isolate belongs to the genus *Penicillium*.

![Figure 2](image1.jpg) **Figure 2.** (A) *Mucor* colonies on PDA medium, (B) Microscopically, (a) sporangiophores (b) sporangium.

![Figure 3](image2.jpg) **Figure 3.** (A) *Penicillium* colonies on PDA, (B) Microscopically, (a) conidia (b) conidiophores.

3.2.4. *Trichoderma*. Two isolates are belonging to the genus *Trichoderma*. Round fungi colonies are greenish white with a dark green circular section. This colony have diameter 3-5.1 cm. The conidiophores branch out and are filled with an oval-shaped conidium (figure 4). Besides being a decomposer, *Trichoderma* also functions as a biological agent and plant growth stimulator. Several species of *Trichoderma* have been reported to provide satisfactory results as biological agents and stimulate plant growth.

![Figure 4](image3.jpg) **Figure 4.** (A) *Trichoderma* colonies on PDA medium, (B) Microscopically, (a) conidia (b) conidiophores.

![Figure 5](image4.jpg) **Figure 5.** (A) *Cladosporium* colonies on PDA medium, (B) Microscopically, (a) conidia (b) conidiophores.

3.2.5. *Cladosporium*. Fungus colonies grow dark green with a diameter of 1-4 cm. Konidia grows thick like a velvety, round shape (figure 5). One isolate belongs to the genus *Cladosporium*. These fungi are usually involved at the beginning of the decomposition process.

3.3. Total fungi

The total number of fungi in *M. indica* leaf litter was 30.35x10³ CFU g⁻¹, whereas in *H. macrophyllus* leaf litter was 33.67x10³ CFU g⁻¹. The fungus population is greater in *H. macrophyllus* leaf litter than *M. indica*. The total number of fungi affects the decomposition process, where the more the number of fungi, the faster the decomposition process will run.

4. Discussion

The process of decomposition of organic matter is influenced by various factors, including substrate quality, carbon availability (C), nutrients and microbial communities. The different types of microbes cause differences in the efficiency of the use of substrate and composition of biomass and the needs of C and nutrients [20]. Fungi is one of the important agents in the process of decomposition of plant biomass and plays an important role in the C cycle in the ecosystem [9, 12].
The results show that five fungi genera successfully isolated from M. indica leaf litter and H. macrophyllus leaf litter. The Aspergillus genus was found in both M. indica and H. macrophyllus leaves. *Aspergillus* sp.1 was found in both leaf litter types, and *Aspergillus* sp.2 was only found in *M. indica* leaf litter. *Aspergillus* sp.3 was successfully isolated from *H. macrophyllus* leaf litter, while *Aspergillus* sp.4 was found in *M. indica* leaf litter and *H. macrophyllus* leaf litter. *Aspergillus* is a fungus that has large adaptability so that it can be found in various ecosystems. Sari et al. [9] also obtained the genus *Aspergillus* from *Salacca zalacca* leaf litter which has the potential to decompose organic materials containing cellulose. Toma and Abdulla [16] also got the genus *Aspergillus* from spices and medicinal plants. Cellulolytic fungi decompose organic matter because they produce cellulase enzymes. Genus *Mucor* and *Penicillium* were found in *H. macrophyllus* leaf litter, *Trichoderma* genus obtained from *M. indica* and *H. macrophyllus* leaf litter, while *Cladosporium* genus was obtained from *H. macrophyllus* leaf litter.

There are differences in the types of fungi isolated from leaf litter *M. indica* and *H. macrophyllus*. The difference in types of fungi is probably caused by differences in the chemical content of leaf litter. All fungi that were decomposers because they were able to form clear zones in CMC medium (specific medium for cellulolytic fungi). Clear zone indicates that fungi produce cellulose enzymes so that they can decompose organic matter.

According to Behera et al. [21], fungi were isolated from the genera *Aspergillus*, *Penicillium*, and *Cladosporium* in addition to *Fusarium*, *Acremonium*, and *Chaetomium*. Isolated fungi were cellulolytic which were able to decompose materials containing cellulose. Okeke et al. [22] isolated lignocellulolytic microbes from biomass pastures and pine, and obtained the genus *Fusarium*, *Penicillium* and *Trichoderma*. This microbe can to decompose litter with cellulose and lignin content. Osono et al. [14] obtained several types of fungi from the leaves of *Salix arctica* namely *Cladosporium*, *Alternaria*, *Phialophora*, and *Venturia*.

Decomposer fungi successfully isolated from *M. indica* leaf litter and *H. macrophyllus* are saprophytic fungi that play a role in the decomposition process of the two leaf litter. The existence of these fungi contributes to the supply of nutrients in the Sei Betung Resort restoration area of the Gunung Leuser National Park so it can accelerate the improvement of soil fertility in the area. Indirectly the presence of decomposer fungi will support plant growth in the restoration area. The results of soil analysis on the restoration area show that although the soil was still acidic with a pH < 5.5, the soil C organic content was included in the medium criteria of 2.39% in the soil under the stand of *H. macrophyllus*, and 2.55% in the soil under *M. indica* stands. It also relates to the ratio of C/ N leaf litter to both stands that are less than 30, so that the decomposition process runs faster and improves soil fertility.

5. Conclusion

There are thirteen decomposer fungi isolates were isolated from leaf litter, namely five isolates from *Macaranga indica* and eight isolates from *Hibiscus macrophyllus* leaf litter. The thirteen isolates belong to six genera namely *Aspergillus*, *Penicillium*, *Mucor*, *Trichoderma*, *Cunninghamella* and *Cladosporium*. Two isolates of *Aspergillus* sp.1, and one isolate of *Aspergillus* sp.2, *Aspergillus* sp.4, and *Trichoderma* respectively were isolated from *Macaranga indica* leaf litter. Two isolates of *Aspergillus* sp.3, two isolates of *Aspergillus* sp.4, and one isolate of *Mucor*, *Penicillium*, *Trichoderma*, and *Cladosporium* respectively were isolated from *Hibiscus macrophyllus* leaf litter.

6. References

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