Growth Response of Kopsia (Kopsia arborea Blume) Plants to Applied Endophytic Fungi

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Abstract. The plant always associates with microbes for its life, includes the endophytes. Aim of this study is to examine the effect of endophytic fungi on Kopsia (Kopsia arborea) seed germination and seedling growth. Here, we used endophytic fungus from forest pathology laboratory, namely Phomopsis sp1, Phomopsis sp2, and Helminthosporium sp. All endophytic fungi were combined with liquid fertilizer to assess Kopsia seedling growth. The results showed that the Kopsia seed germinations, which treated with endophytic fungi have a higher value than the control. The endophytic fungi Phomopsis sp1 and Helminthosporium sp could increase the number of leaves without adding liquid fertilizer, while the Phomopsis sp2 needed liquid fertilizer for it. The NPA and the IMB values of the Kopsia seedlings are 0.71–1.71, and 0.18–0.33 strongly indicates the readiness of Kopsia seedlings to plant in the field, respectively.

1. Introduction

Kopsia (Kopsia arborea Blume) is one of the forestry plants that function as a pedestrian shelter and can add aesthetic value through beautiful white flowers when in bloom [1]. Kopsia meets the requirements as a shade plant, then the fruit is not too big, not shed many leaves, sturdy trees, root growth is not too fast, and spared from pests and diseases [2]. Based on the morphology, Kopsia are suitable plants planted in green open spaces. This plant can grow in various types of soil and grow at an altitude of 1500 meters above sea level [3].

Aside from being an ornamental plant or shade plant, Kopsia is used as a medicinal plant because there is secondary metabolite content in it. Kopsia is a type of plant from the Apocynaceae family which has the potential as a source of natural antioxidants. The results showed that in Kopsia fruit extracts, there were secondary metabolites of flavonoid, saponin, tannin, and alkaloid compounds [4]. Kopsia plants have not been so popular in Indonesia and have not been extensive research for cultivation. Plant cultivation is inseparable from disturbances that can inhibit plant growth.

Some biological agents are known to increase plant growth, namely endophytic fungi [5]. The ability of endophytic fungi to enhance plant growth depends on its ability to produce some secondary metabolites of growth regulator. Each type of endophytic fungi has different skills to help plant growth. Endophytic fungi as biological agents in addition to increasing plant growth, can also reduce nematode infections, increase plant resistance to stress, and produce secondary metabolites [6].

The addition of liquid fertilizer in planting medium can make nutrient absorption by endophytic fungi more effective. However, not all of the endophytic role in increasing plant growth is known, except for some species that can produce phytohormones such as ethylene, auxin, and cytokinins [7] or increase the ability of plants to absorb nutrients [8]. Therefore, it is necessary to research the
treatment of endophytic fungi on the growth of Kopsia. Aim of this study is to examine the effect of endophytic fungi on Kopsia (Kopsia arborea) seed germination and seedling growth.

2. Method

2.1. Materials
The tools used in this study were poles, knives, plastic germinations, polybags 15 cm x 20 cm, scales 0.01 g, oven, autoclave, laminar airflow, petri dish, mortar, plastic container, sprayer, callipers, ruler, and camera. The materials used in the study were Kopsia seeds, endophytic fungi from the Forest Pathology Laboratory collection (Phomopsis sp1, Phomopsis sp2, and Helminthosporium sp), 70% alcohol, sterile water, potato dextrose agar (PDA) medium, and liquid fertilizer. The medium for seed germination barren sand and the medium for weaning are soil, cocopeat, husk charcoal at a 6: 3: 1 (v / v / v) ratio.

2.2. Procedure
The study consisted of two experiments, (1) application of endophytic fungi on Kopsia seeds and (2) application of endophytic fungi and liquid fertilizer on Kopsia seedlings. Both tests were carried out in a greenhouse.

The preparation phase is the downloading of Kopsia and the propagation of endophytic fungi. The fruit is given a pretreatment for breaking dormancy by cutting the tip of the fruit (scarification). Endophytic fungi (Phomopsis sp1, Phomopsis sp2, and Helminthosporium sp) planted on PDA medium. After 14 days old, mycelium suspension (20 mL/petri dish) used in this study.

The first experiment, seeds were soaked in a suspension of endophytic fungi for 24 hours, whereas for control it was only soaked using sterile water. Then, the seeds germinated in barren sand. The study used a completely randomized design with one factor, endophytic fungi (3 levels). The endophytic fungi used in this study is Phomopsis sp1, Phomopsis sp2, and Helminthosporium sp. Each level consists of three replications, which consists of 50 Kopsia seeds. Seeds were grown in a greenhouse for 3 weeks. Percentage of germination, the rate of germinations, maximum growth potential, and the increasing speed were determined three weeks after seed planting.

The second Experiment, the Kopsia seeds are germinated in sterile sand. Four weeks old after sowing, root seedlings were soaked in a suspension of endophytic fungi for 24 hours, whereas for control only are soaked using sterile water. Treated seedlings planted in 15 cm x 20 cm polybag that contained growing media (soil, cocopeat, and sterile husk charcoal at a 6: 3: 1 (v / v / v) ratio). Transplant the seedlings is carried out in the afternoon and keep transplanted seedlings in the shade. Liquid fertilizer (10 mL/L) was applied to both treated and control seedlings four times during this study. The study used a complete randomized design with a factorial pattern consisting of two factors i.e., endophytic fungi and liquid fertilizer. The endophytic fungi (3 levels) used in this study, i.e., Phomopsis sp1, Phomopsis sp2, and Helminthosporium sp. Biological liquid fertilizer consists of two levels, i.e., treated and control.

Seedlings height were measured every week from the 1st to the 16th. These parameters allowed us to assess the plant vigor over the growing period. Diameter and the number of leaves were also measured every four weeks. At the end of the study, some plant growth parameters such as total wet weight (g), total dry weight (g), plant moisture content (%), root shoot ratio (NPA), and seedlings quality index (IMB) determined. Shoots and roots were separated, dried at 80 °C until constant weight (about 72 hours), and weighed for biomass.

For each dependent variable, data were analyzed using analysis of variance procedures in Statistical Analysis Software (portable SAS 9.1.3). Duncan Multiple Range Test (DMRT) value were given to differentiate the treatments.

3. Result and discussion
Plants are always associated with microbes, including endophytes in their lives. Some endophytes are reported to be able to increase growth and suppress pathogenic activity in plants. Endophytic fungi live in plant tissues for a certain period and able to form colonies in the tissue without endangering the host [9]. Each plant can contain one or more endophytic microbes consisting of fungi or bacteria [10]. Endophytic fungi live intracellular in healthy plant tissue and induce the host to produce secondary metabolites. This induction can be caused by genetic recombination or coevolution [11].

In this study, endophytic fungi used, isolated from kemaitan or sanrego plants (Lunasia amara Blanco) with the species are Phomopsis sp1, Phomopsis sp2, and Helminthosporium sp. All of the isolates used in the study tested for their pathogenicity, and the test results showed that the strains were non-pathogenic fungi so that it could be inoculated in other plants and did not cause disease in the inoculated plants, so all of these isolates classified as neutral or beneficial endophytes.

The treatment of endophytic fungi can accelerate the germination of Kopsia seeds. Kopsia seeds treated with endophytic fungi began to germinate on the first day after planting, while those not given endophytic fungi (control) began to grow on the sixth day after planting. The results of variance (Table 1) showed that endophytic fungi only significantly affected the value of Kopsia seed germination. The control and those treated with endophytic fungi all grew, only the germination of those treated with endophytic fungi developed earlier than controls.

| Variable               | Endophytic fungi |
|------------------------|------------------|
| Percentage of germination | 0.1397<sup>n</sup> |
| Germination rate        | 0.3267<sup>n</sup> |
| Germination value       | 0.0336<sup>*</sup> |
| Maximum growth potential | 0.1112<sup>n</sup> |
| Growing Speed           | 0.0730<sup>n</sup> |

<sup>n</sup> = not significantly different, <sup>*</sup> = significantly different at the 95% confidence level

The treatment of endophytic fungi can accelerate the germination of Kopsia seeds. Kopsia seeds treated with endophytic fungi began to germinate on the first day after planting, while those not given endophytic fungi (control) began to grow on the sixth day after planting. Germination is a process that reactivates the growth activity of the embryonic axis in the stalled seed to form seedlings [12]. The response of Kopsia germination, which was treated by endophytic fungi for three weeks, only had a significant effect on the germination rate variable. Its because the seeds treated with endophytic fungi germinated earlier in the first week compared to controls that began to germinate a lot in the third week.

According to [13], the percentage of normal seed germination provides information related to the ability of seeds to grow into plants that produce natural results. The results obtained indicate that the three endophytic fungi did not affect the germination of Kopsia seeds. The percentage of seed germination obtained was ± 76%. This result improved by maintaining the moisture of the growing medium. In this study, planting medium that was too moist caused ± 20% of Kopsia seeds to rot.

The rate of germination can be measured by counting the number of days needed for the appearance of radicles or plumule [13]. Based on the value of germination rate, treatment of endophytic fungi has a faster germination rate of Kopsia compared to control. This is because the Kopsia germination grows earlier. However, the value of the germination rate of Kopsia seeds treated with endophytic fungi was more diverse, while in the control treatment, the seed germination rate was more evenly distributed in its growth.

The germination value is the percentage of seeds that germinate every day, so it has a relation with the rate of germination. If the germination rate only shows the average day of germination, the germination value indicates the number of germinated seeds as a percentage every day until the end of
the test [14] which is a reflection of the growing capacity of the grain. The results showed that the treatment of endophytic fungi (Phomopsis sp1, Phomopsis sp2, Helminthosporium sp) could increase the value of Kopsia seed germinations. The seeds given by endophytic fungi germinated a lot at the end of the first week, while in control many germinated in the third week.

Table 2 The germination value of Kopsia germination treated with endophytic fungi

| Endophytic fungi | Germination value | Improvements to controls (%) |
|------------------|-------------------|------------------------------|
| Kontrol          | 2.29b             | 0.00                         |
| Phomopsis sp1    | 5.00ab            | 118.47                       |
| Phomopsis sp2    | 6.87a             | 199.86                       |
| Helminthosporium sp | 5.69a         | 148.60                       |

The number followed by the same letter indicated that the treatment was not significantly different at the 95% confidence level.

The treatment of endophytic fungi Phomopsis sp2 and Helminthosporium sp results in the best of the value of germinations with a percentage increase against the control of 199.86% and 148.60%. The treatment of endophytic fungi of Phomopsis sp1 also increases the value of germination of Kopsia seed germination because it has a percentage increase in control of 118.47% although the value is lower than that of endophytic fungi of Phomopsis sp2 and Helminthosporium sp (Table 2). It indicates that the treatment of endophytic fungi can increase the value of Kopsia seed germinations.

The maximum growth potential (PTM) is the total number of life seeds or shows life symptoms [15]. In all treatments, including control has seeds that will germinate usually or abnormally, the values tend to be the same. The seeds obtained from the tree that had met the criteria as a seed tree.

The requirements for the seed tree are the age of the tree is old and has been fruiting at least three times, has a functional appearance such as a lush canopy and large tree trunks and is free from pests and diseases [16].

Speed of germination is the speed of seed to germinate, can be calculated by counting the number of days required for the appearance of radicles and plumule [13]. The speed of germination is related to the rate of imbibition by the seed coat. The thicker of seed coat will take, the longer it will bring water to fill the cavity of the seed coat. This condition will cause a slow germination [17]. The Kopsia seed used in this study was given a preliminary treatment (scarification at the tip of the seed coat) to accelerate the seed imbibition process.

Kopsia seeds treated with endophytic fungi can grow faster than controls, but the standard deviation value in the treatment of endophytic fungi is higher than that of controls so that they are not significantly different.

![Figure 1. Growing speed in the germination of Kopsia treated with endophytic fungi](image-url)

In the seed experiment, the percentage of live Kopsia seedlings used in this study was 100%, and all seedlings performed well. This shows that the endophytic fungi used are non-pathogenic. The
results showed that the combination of treatment of endophytic fungi and liquid fertilizer could increase the number of Kopsia seedling leaves. The presence of leaves in plants can be related to the ability of the plant to photosynthesize and produce photosynthates in the form of carbohydrates needed by plants for growth and development [18]. Leaves have an essential role in the primary productivity of plants [19]. Not all types of endophytic fungi used in this study require the addition of liquid fertilizer to increase the number of Kopsia leaves. *Phomopsis* sp1 and *Helminthosporium* sp do not require the addition of liquid fertilizer, whereas *Phomopsis* sp2 require the addition of liquid fertilizer to increase the number of leaves in the Kopsia seedlings.

The application of endophytic fungi and liquid fertilizer did not affect the growth of Kopsia seedlings in the treatment of single endophytic fungi or single liquid fertilizer. This can be seen in the variables of height, diameter, total wet weight (BBT), total dry weight (BKT), increase in the number of leaves, and water content of plants which show the results of the treatment are not significantly different. The opposite occurred in the treatment of interactions between endophytic fungi and liquid fertilizer which showed significantly different effects on the variable number of leaves (Table 3).

**Table 3** Recapitulation of the growth of Kopsia seedlings which were given fungus and liquid fertilizer

| Variable                  | Treatment                     | Endophytic fungi (C) | Liquid fertilizer (P) | C × P   |
|---------------------------|-------------------------------|----------------------|----------------------|---------|
| Height                    |                               | 0.7212<sup>m</sup>   | 0.8900<sup>m</sup>   | 0.8612<sup>m</sup> |
| Diameter                  |                               | 0.3525<sup>m</sup>   | 0.7462<sup>m</sup>   | 0.4880<sup>m</sup> |
| Total wet weight (BBT)    |                               | 0.2060<sup>m</sup>   | 0.4462<sup>m</sup>   | 0.6042<sup>m</sup> |
| Total dry weight (BKT)    |                               | 0.1959<sup>m</sup>   | 0.3774<sup>m</sup>   | 0.7613<sup>m</sup> |
| Number of leaves          |                               | 0.9110<sup>m</sup>   | 0.0596<sup>m</sup>   | 0.0171*      |
| Water content             |                               | 0.0922<sup>m</sup>   | 0.3939<sup>m</sup>   | 0.5964<sup>m</sup> |

<sup>m</sup> = not significantly different, * = significantly different at the 95% confidence level

The addition of liquid fertilizer on the planting medium can interact positively or negatively on endophytic fungi. For example, *Helminthosporium* sp and *Phomopsis* sp1 do not require the addition of liquid fertilizer to increase the number of Kopsia leaves, whereas the *Phomopsis* sp2 involves the addition of liquid fertilizer to increase the number of Kopsia leaves (Table 4).

**Table 4** The interaction between the treatment of endophytic fungi and liquid fertilizer on the increasing number of leaves

| Endophytic fungi | Liquid fertilizer | Increase (%) | Increase (%) |
|------------------|-------------------|--------------|--------------|
|                  | 0 (mL)            | 10 (mL)      |              |
| Control          | 3.7<sub>ab</sub>  | 3.3<sub>ab</sub> | -9.1        |
| *Phomopsis* sp1  | 4.2<sub>a</sub>   | 2.6<sub>b</sub> | -27.3       |
| *Phomopsis* sp2  | 3.2<sub>b</sub>   | 4.2<sub>a</sub> | 14.5        |
| *Helminthosporium* sp | 4.2<sub>a</sub> | 2.9<sub>b</sub> | -20.9       |

The number followed by the same letter indicated that the treatment was not significantly different at the 95% confidence level

The number of Kopsia seedlings treated with endophytic fungi increased by ± 15% compared to controls. However, the increasing number of leaves has not been accompanied by an increase in height, diameter, and biomass of Kopsia seedlings. The value of biomass is related to plant metabolism or plant growth conditions for the ongoing metabolic activity of plants such as photosynthesis. Thus the higher biomass shows the photosynthesis process to take place more efficiently [20].
Figure 2  Kopsia seed biomass: a. Total wet weight (BBT) and total dry weight (BKT), b. Water content in Kopsia seeds. E0: without endophytes, E1: Phomopsis sp1, E2: Phomopsis sp2, E3: Helminthosporium sp, F0: without liquid fertilizer, F1: with liquid fertilizer.

The treatment of endophytic fungi and liquid fertilizer has not been able to increase the biomass of Kopsia seedlings (Figure 2a). It can be seen from the test results showed no significant difference in BBT and BKT variable for all treatments. Test results that were not significantly different were also found in the water content variables of Kopsia seedlings (Figure 2b). The NPA (root shoot ratio) and IMB (seed quality index) describe the quality of seedlings based on their adaptability to the environment in which they are growing. NPA value is a seedling growth variable that compares dry shoot weight (stem and leaf) with root dry weight. The NPA value is used to find out the plant balance of the upper part of the plant as a place for transpiration and photosynthesis with the roots as the absorption area [21]. Kopsia seedlings in all treatments had NPA values around 0.71-1.17.

According to [22] seeds that meet the criteria are seedlings with a root shoot ratio of 1–2. The lower 0f NPA value, then the better quality of seedlings. If plant growth is average, NPA values close to 1 indicate good plant growth in Kopsia seedlings. NPA value criteria depend on the type of seed. Good seedlings are seeds that have balanced growth between the top and the roots of plants and for each type of plant have different NPA values [23]. The results showed that the Kopsia seedlings had an IMB value > 0.09 for all treatments, which ranged 0.18–0.33. IMB value > 0.09 indicates that the seedlings are declared suitable for planting in the field [24]. The higher the IMB value, the higher the quality of seedlings. Based on NPA and IMB values, Kopsia seedlings have good adaptability if transferred to the field [25].
Figure 3. Performance of Kopsia seedlings after 16 weeks of treatment E0: without endophytes, E1: Phomopsis sp1, E2: Phomopsis sp2, E3: Helminthosporium sp, F0: without liquid fertilizer, F1: with liquid fertilizer

Figure 4. Kopsia seed value: a. Root shoot ratio (NPA), b. Seed quality index (IMB). E0: without endophytes, E1: Phomopsis sp1, E2: Phomopsis sp2, E3: Helminthosporium sp, F0: without liquid fertilizer, F1: with liquid fertilizer

NPA and IMB values in Kopsia seedlings in this study indicate that all Kopsia seedlings have excellent seed performance to be planted in the field (Figure 3). An assessment of the ability of a seedling to be ready for transfer to the area can be seen from the NPA and IMB values. Kopsia seedlings in all treatments had NPA values from 0.71−1.17 (Figure 4a), while IMB values ranged from 0.18−0.33 (Figure 4b).

4. Conclusion
The treatment of endophytic fungi on Kopsia seeds can increase the value of seed germinations; whereas in Kopsia seedlings, endophytic fungi can increase the number of leaves. Endophytic fungi did not dominate in increasing the germination of Kopsia seeds. However, the growth of seedlings (increasing the number of leaves) obtained results of *Phomopsis* sp1, and *Helminthosporium* sp can increase the number of leaves without the addition of liquid fertilizer, whereas *Phomopsis* sp2 requires liquid fertilizer to increase the number of Kopsia leaves. Kopsia seeds have NPA values around 0.71−1.17, IMB around 0.18−0.33, and excellent performance that meets the criteria that the plant planted in the field.

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