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Effect of Endophytic Fungi on the Growth of Sengon (*Falcataria moluccana*)

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ABSTRACT

Endophytic fungi have been reported to promote plant growth and resistance to biotic and abiotic stresses. This study aimed to determine the effect of endophytic fungi application isolated from the sengon (*Falcataria moluccana*) seeds on the growth of sengon. The seed-borne endophytic fungi were applied when the session was three weeks old by sprinkling each suspension of endophytic fungi (10^3 to 10^6 spores/ml) on the soil around the plant roots. The experimental parameters evaluated were height, diameter, and total wet and dry weight. This study showed that endophytic fungi treatment has no significant effect on the stem diameter, total wet weight, and water content; but significantly affected the height and total dry weight. *Cladophialophora* sp. and *Ascotricha* sp. fungi increased the height growth of sengon, while *Aspergillus* sp. fungi increased the total dry weight against the control. *Ochroconis* sp. treatment was not significantly different from the control. The shoot-root ratio values range from 1.13-1.43, indicating that the seeds already have roots supporting plant growth. The Dickson quality index range from 0.65-1.03 suggested that the seedlings have good biomass distribution. Consequently, endophytic fungi have the potential to increase the growth of sengon seedlings.

1. Introduction

Sengon (*Falcataria moluccana*) is one type of plantation species that potentially meets timber needs. The production of sengon wood has increased from 3,651,479 m^3 in 2018 (BPS 2018) to 5,468,716 m^3 in 2019 (BPS 2019). Sengon is a fast-growing species capable of substituting wood materials widely used in reforestation and restoration programs (Nugroho et al. 2021). In addition, sengon leaves can be used as animal feed because they contain high protein (Hartanto 2011). However, sengon plants are susceptible to gall-rust and damping-off.

Gall-rust disease can attack sengon at various stages of growth. Lestari et al. (2013) reported that the gall-rust disease decreased the productivity of sengon. Other symptoms often found in nurseries are damping-off that causes severe damage, decay, and seedling death (Istikorini and Sari 2020). Disease attacks at the seedling level will affect the quality and quantity of seedlings. Pest and disease resistance and the growth rate of sengon seedlings are crucial factors in meeting the increasing demand for wood (Putri and Bramasto 2017). One of the efforts that potential is by utilizing biological agents.

The use of biological agents such as endophytic fungi in the forestry sector is increasing. Endophytic are fungi that inhabit internal plant tissue without causing damage to the host (Rashmi et al., 2019). Endophytic fungi improved plant growth and resistance to biotic and abiotic stresses (Kumar and Radhakrishnan 2020). The research by Rasyad et al. (2019) showed that the endophytic fungus from the kemaitan plant (*Lunasia amara*) could increase the germination of kopsia (*Kopsia arborea*) seeds. Hermawan (2019) showed that the endophytic fungi significantly affect the growth rate of sengon's sprouts. In addition, endophytic fungi can produce alkaloids and mycotoxins that are toxic to pathogens (Singh et al., 2011).

Endophyte fungi have improved plants' growth and resistance to pests and disease. The research conducted by Istikorini et al. (2021) showed several types of endophytic fungi found inside the seeds of sengon, including *Ochroconis* sp., *Cladophialophora* sp., *Ascotricha* sp., and *Aspergillus* sp., which were non-pathogenic (Istikorini et al. 2022). However, the information related to these endophytic fungi to improve plant growth is still lacking. Therefore, research on the effect of endophytic fungi on the growth of sengon needs to be observed. This study aimed to determine the effect of endophytic fungi application isolated from seeds of sengon on the growth of sengon. The result from this study would provide information on the effect of seed-borne endophytic fungi on the growth of sengon and help the local farmers optimize timber yields. In addition, the government can take advantage of additional information related to developing sengon plants to meet the demand for wood.

2. Materials and Methods

2.1. Materials

The materials used in this study were sengon seeds (*Falcataria moluccana*), as many as 150 seeds, and isolates of the seed-borne endophytic fungi from the sengon (*Ochroconis* sp., *Cladophialophora* sp., *Ascotricha* sp., and *Aspergillus* sp.).

2.2. Methods

2.2.1. Sengon seed preparation

Sengon seeds used are 150 selected by criteria of clean skin, dark brown, uniform seed size, sink in water when soaked, and the seed shape is still intact (Nabu and Taolin 2016). Breaking the dormancy of sengon seeds was done by soaking them in 80^{o}C water for 15 min, then soaking them in room temperature water for 24 h (Aurum et al. 2020).

2.2.2. Growing media preparation

The media used for seed germination and weaning was a mixture of goat manure and soil with a ratio of 1:3 (w/w). Goat manure contains potassium (K) relatively higher than other manures (Sanjaya et al., 2021). Potassium is an activator of several enzymes in plant metabolism, such as protein synthesis, sugar transport, nitrogen (N), and carbon (C) metabolism, which increases plant resistance to disease (Xu et al. 2020). The soil was sterilized using an autoclave at 121°C and 1 atm for 1 h. Then, seed germination was carried out in a germination container filled with 1/3 of the mixed media for two weeks. After two weeks, the seeds were weaned using polybags 10 cm × 15 cm containing the media of 400 g of a mixture of goat manure and soil.

2.2.3. Rejuvenation and propagation of endophytic fungi

The endophytic fungi used in this study were isolated from the seeds of sengon, namely *Ochroconis* sp., *Cladophialophora* sp., *Ascotricha* sp., *and Aspergillus* sp. (Istikorini et al. 2021). Isolates of endophytic fungi were rejuvenated and propagated on Potato Dextrose Agar (PDA) medium and then incubated for 14 days at room temperature. Endophytic fungi that have grown are made into spore suspensions, and then the spore density is calculated using a hemacytometer.

2.2.4. Weaning and seedling maintenance

Sengon seedlings are weaned after 14 days old. Seedlings ready for weaning are characterized by the appearance of at least 2 compound leaf stalks that grow and develop healthily (Krisdayani et al. 2020). Weaning is done by preparing a polybag containing weaning media and then watering it first so that the media is moist. The maintenance of sengon seedlings was carried out during the observation period or a month, including watering in the morning or evening, cleaning weeds, and controlling pests and diseases mechanically.

2.2.5. Application of endophytic fungi

Endophytic fungi were applied one week after the plants were weaned into polybags. Endophytic fungi were applied by sprinkling each suspension of endophytic fungi (about 10^3 to 10^6 spores/ml) around the plant roots as much as 1 ml. According to (Magdalena et al. 2021), the application of endophytic fungi is often carried out by soaking the seeds or spraying the suspension on the soil.

2.3. Data Collection

Data collection was carried out for four weeks or a month after applying endophytic fungi. The observed variables are described below.

2.3.1. Seedling height

Measurement of stem diameter was carried out at the first and the end of the application. Stem diameter measurement was carried out at 1 cm above the stem base using a digital caliper. The rod diameter value is expressed in millimeters (mm).

2.3.2. Stem diameter

Measurement of stem diameter was carried out at the first and the end of the application. Stem diameter measurement was carried out 1 cm above the stem base using a digital caliper. The rod diameter value is expressed in millimeter (mm).

2.3.3. Total wet weight

The total wet weight was measured at the end of the observation. The shoots and roots of the plants were separated, and then the wet weight of each part of the plant was measured. Total wet weight is the sum of the wet shoot and root wet weights. The total wet weight value is expressed in grams (g).

2.3.4. Total dry weight

The total dry weight measurement was carried out at the end of the observation. The shoots and roots of the plants were separated and then dried using an oven at 105°C for 24 h. After that, each plant was weighed (Tefa et al. 2015). Total dry weight is the sum of the shoot and dry root weights. The total dry weight value is expressed in gram (g).

2.3.5. Plant water content

The water content measurement was carried out at the end of the observation using the following formula (Manurung et al. 2013):

$$Water\ content\ (\%) = \frac{Total\ wet\ weight\ (g) - Total\ dry\ weight\ (g)}{Total\ wet\ weight\ (g)} \times 100\% \quad (1)$$

2.3.6. Shoot-root ratio

The shoot-root ratio (SRR) measurement was carried out at the end of the observation and can be formulated as follows (Wang et al. 2020):

$$SRR = \frac{Shoot\ dry\ weight\ (g)}{Root\ dry\ weight\ (g)} \quad (2)$$

2.3.7. Dickson quality index

Dickson quality index (DQI) measurement was carried out at the end of the observation and calculated using the following formula (Kuniarty et al. 2010):

$$DQI = \frac{Total\ wet\ weight\ (g)}{\frac{Shoot\ wet\ weight\ (g)}{Root\ wet\ weight\ (g)} + \frac{Seed\ height\ (cm)}{Stem\ diameter\ (mm)}} \quad (3)$$

2.4. Experimental Design

The research design used was a completely randomized design (CRD) with one factor. The factor was the type of endophytic fungi, which consists of 5 levels (*Ochroconis* sp. *Cladophialophora* sp. *Ascotricha* sp. *Aspergillus* sp., and control). Each treatment was repeated five times.

2.5. Data Analysis

The analysis of variance was conducted to determine the effect of the treatment given. The study was analyzed using IBM SPSS Statistics 25. If the P-value > α (0.05), the effect of treatment on the observed variables is not significant. If the P-value < α (0.05), the effect of treatment on the experimental variable is statistically significant and will be followed by Duncan's Multiple Range Test (DMRT). The SRR value refers to the classification of Duryea and Brown (1984), stating that the best seedling viability generally occurs at an SRR between 1-3, and the best is closer to the minimum value. Meanwhile, the DQI refers to Azzahro et al. (2020), which stated that the best seedlings have a DQI value > 0.09. The higher the DQI, the better the seedling quality.

3. Results and Discussion

3.1. Effect of Endophytic Fungi on the Growth of Sengon

Growth in plants occurs when the volume or mass of cells increases. Plant growth is influenced by genetic factors possessed by a plant and external factors or environmental factors (Kuswandi and Sugiarto 2015). One of the efforts to improve plant growth is utilizing endophytic fungi. **Table 1** shows the growth responses of sengon with the application of seed-borne endophytic fungi.

Table 1. Analysis of variance of the effect of seed-borne endophytic fungi on the growth of sengon seedlings

Parameters	P-value
Seedling height (cm)	0.000*
Stem diameter (mm)	0.628^{ns}
Total wet weight (g)	0.127^{ns}
Total dry weight (g)	0.003*
Water content (%)	0.749^{ns}

Notes: The numbers in the table are significant values. ns= no significant effect. *= treatment has a significant effect at 5% test level with P-value < 0.05.

The analysis of variance showed that the treatment of endophytic fungi significantly affected the seedling's height and total dry weight at the 5% test level (**Table 1**). Meanwhile, the treatment of endophytic fungi did not significantly affect the plant diameter, total wet weight, and water content at the 5% test level. Mejia et al. (2013) stated that plants respond to various microbes, including endophytes, both genetically and physiologically. Therefore, the effect of endophytic fungi infection on plants will differ depending on the type of plant and the age or stage of plant development from seedlings to mature plants (Yuniarti 2013). Research conducted by Ramdan et al. (2013) on chili seedlings showed different results; endophytic fungi indicated neutral properties to seedling growth, promoting seedling growth or inhibiting chili seed growth compared to controls.

3.2. Seedling Height

Height is primary growth that occurs in the meristems at the tips of roots and shoots. Primary growth allows roots to make connections in the ground and shoots to increase exposure to sunlight and carbon dioxide (Meiganati and Rusli 2017). Thus, plant height is a growth indicator used to measure the environment's effect or treatment (Sulardi and Sany 2018).

The results of Duncan's Multiple Range Test (DMRT) show that the endophytic fungi treatment has a better effect on plant height growth than the control (**Table 2**). Treatment of *Ascotricha* sp. and *Cladophialophora* sp. increased the seedling height with a percentage increase of 135.27% and 85.19% against the control. Research conducted by Ramdan et al. (2013) showed that endophytic fungi could increase the height growth of chili seedlings. Research conducted by Irawati et al. (2014) also showed that the application of endophytic fungi gave better height growth than controls on rice plants. Treatment of endophytic fungi can probably improve root performance. The roots can absorb more nutrients and water, which affects the seedling height.

Endophytic fungi's ability to increase plant growth depends on their ability to produce growth-promoting metabolites (Ramdan et al. 2013). Syamsia (2016) showed that endophytic fungi could produce auxin hormones essential in cell elongation. Plants need auxin hormone in optimum amounts to help root elongation, and if the levels are higher, it can inhibit root elongation (Haryani et al. 2019). According to Irawati et al. (2017), endophytic fungi can produce secondary metabolites allegedly due to taking genetic information from their host in the host plant tissue.

Table 2. The effect of seed-borne endophytic fungi on the height growth of sengon seedlings

Treatment	Seedling height
Control	4.77[c]
Ochroconis sp.	6.80[bc]
Cladophialophora sp.	8.83[ab]
Ascotricha sp.	11.22[a]
Aspergillus sp.	6.48[bc]

Notes: The numbers followed by the same letter show that the treatment is not significantly different at the 5% test level.

3.3. Stem Diameter

Diameter is one of the essential parameters to see the growth of a plant. Diameter growth occurs if the needs of photosynthesis for respiration, leaf replacement, root growth, and plant height have been met (Shoda et al. 2020; Suryani et al. 2018). The treatment of endophytic fungi did not significantly affect stem diameter at the 5% test level. Sengon treated with seed-borne endophytic fungi tended to have a higher diameter growth than the control. Treatment of *Aspergillus* sp. and *Cladophialophora* sp. can increase stem diameter growth with a percentage increase of 8.89% and 8.86% against the control (**Fig. 1.**).

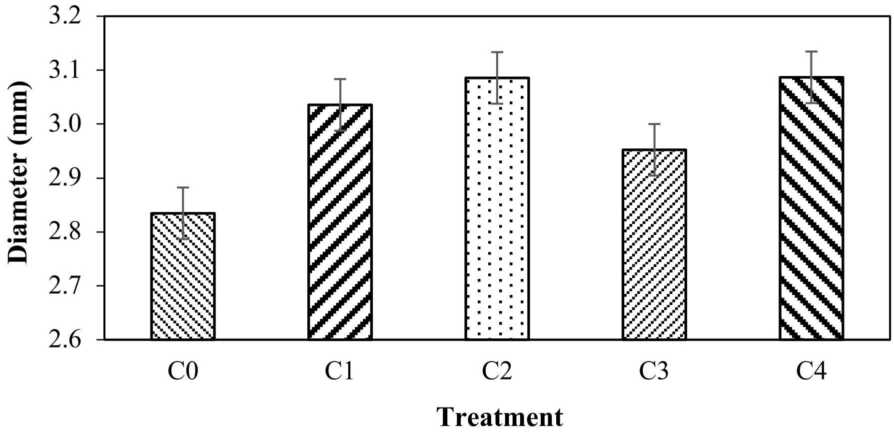

Fig. 1. The effect of seed-borne endophytic fungi on the stem diameter growth of sengon. C0: Control, C1: *Ochroconis* sp., C2: *Cladophialophora* sp., C3: *Ascotricha* sp., C4: *Aspergillus* sp.

The effect of endophytic fungi treatment on the diameter growth was not significant. This might be caused by the age of sengon seedlings being too young, so the diameter was not optimally grown. This tendency is supported by Puspadewi et al. (2016), who stated that the absorption of nutrients by plants in the seedling phase will focus on height growth and will be absorbed for diameter growth when approaching the near end of the vegetative phase. According to Campbell et al. (2012), the seedling phase prioritizes shoot and root growth because it has a fundamental function for plant growth and development in the long term, such as diameter.

3.4. Sengon Biomass and Water Content

The total wet weight is the weight of the plant that shows the accumulation of plant metabolic products during the growth period. Meanwhile, the total dry weight shows the accumulated organic compounds stored in all plants after dried water (Rajak et al. 2016). Sufficient water and nutrients will support plant growth. The nutrients absorbed by the roots will be used to synthesize organic compounds in the form of ions in plant tissues and increase plant dry weight (Rastono and Marsusi 2018). Treatment of seed-borne endophytic fungi significantly affected the total dry weight but did non-significantly affect the total wet weight and water content (**Table 3).**

Table 3. The effect of seed-borne endophytic fungi on the biomass and water content of sengon seedlings

Treatment	Parameter		
	TTW (g)	TDW (g)	Water content (%)
Control	18.22[b]	4.27[a]	75.92[a]
Ochroconis sp.	20.84[ab]	4.76[a]	76.41[a]
Cladophialophora sp.	19.97[b]	4.46[a]	77.34[a]
Ascotricha sp.	20.56[ab]	4.61[a]	77.24[a]
Aspergillus sp.	26.15[a]	6.25[b]	76.21[a]

Notes: The numbers followed by the same letter in the same column indicate that the treatment is not significantly different at the 5% test level. TWW = total wet weight, TDW = total dry weight.

The treatment of seed-borne endophytic fungi tended to increase the total wet weight and total dry weight of sengon. Kumar and Radhakrishnan (2020) stated that the endophytic fungi could increase the total wet weight of plants. Sufficient water and nutrients can support the photosynthesis process optimally to produce photosynthate for plant growth and development (Rahmah et al., 2014). In addition, fungal colonization on roots generally changes the root system, increasing plant weight (Ramadhan et al. 2017).

Treatment of *Aspergillus* sp. gave the most significant increase in total wet weight and total dry weight of sengon with a percentage increase of 43.51% and 46.34% compared to the control. Treatment *Aspergillus* sp. is significantly different from other treatments in increasing the total dry weight but did not significantly different in increasing the total wet weight (**Table 3**). Treatment of Aspergillus sp. is allegedly capable of increasing the available nutrients better than other treatments. Artha et al. (2013) state that *Aspergillus* sp. is a phosphate solubilizing fungus that can dissolve phosphate compounds available to plants. Phosphate plays a role in cell division to form plant organs. High phosphate nutrients stimulate root growth (Neoriky et al., 2017). If the phosphate nutrients are sufficient and can fulfill the needs of plant growth, it can cause an increase in biomass.

3.5. Shoot-Root Ratio and Dickson Quality Index

Shoot-root ratio (SRR) is a parameter of seedling growth that compares plant tissues with a growth function with tissues with a supporting role. The Dickson quality index (DQI) shows the morphological characteristics by calculating the resistance and distribution of biomass. The SRR and DQI values of sengon treated by endophytic fungi are listed in **Table 4.**

Table 4. The effect of seed-borne endophytic fungi on the shoot-root ratio and Dickson quality index of sengon seedlings

Treatment	Parameter	
	SRR	DQI
Control	1.25	0.77
Ochroconis sp.	1.43	0.79
Cladophialophora sp.	1.13	0.65
Ascotricha sp.	1.18	0.63
Aspergillus sp.	1.21	1.03

Notes: SRR = shoot-root ratio, DQI = Dickson quality index.

The SRR value was intended to show the morphological balance within the seedling to absorb water through the roots and resupply the aboveground portion as moisture is lost through evapotranspiration (Sheridan et al., 2021). Duryea and Brown (1984) stated that good seedlings had SRR values ranging from 1–3. Sengon seedlings had SRR values ranging from 1.13–1.43 (**Table 4**), indicating that sengon seedlings had a balanced growth between the shoots and roots. The SRR value is closer to 1, indicating that the seeds already have roots supporting plant growth (Ramadhan et al., 2018). A higher SRR value indicating the availability of water and nutrients is relatively optimal. As a result, the growth of the shoot will be more dominant.

Meanwhile, a smaller SRR value indicates that the available water and nutrients are relatively low. As a result, root development will be more dominant in increasing plant water and nutrient uptake (Wijayanto and Kardiyono 2020). Ban et al. (2017) research showed that plants inoculated with endophytic fungi increased the shoot-to-root biomass ratio compared to non-inoculated plants. Furthermore, it indicated that the inoculated plants invested more biomass in root production.

The DQI value reflects the dry weight that indicates whether the plant is good or not and is closely related to the availability of nutrients. Sufficient nutrients can support plant survival (Sembiring et al., 2013). Sengon seedlings in all treatments had DQI values > 0.09, which ranged from 0.65–1.03. Research conducted by Rasyad et al. (2019) also showed that the application of endophytic fungi on kopsia (*Kopsia arborea* Blume) resulted in a DQI value > 0.09. DQI value > 0.09, indicating the seedlings have better survival (Azzahro et al. 2020). The highest DQI value was obtained from the treatment of the fungus *Aspergillus* sp., which has the highest total dry weight value. Harimurti et al. (2015) state that the total dry weight influences the DQI. The higher the total dry weight value, the higher the DQI value.

4. Conclusions

The seed-borne endophytic fungi treatment could significantly increase the seedling height and total dry weight. However, it has no significant effect on the stem diameter, total wet weight, and water content. In general, the seeds treated with seed-borne endophytic fungi tended to have a higher value than the control on all parameters. The highest height growth was obtained from the *Ascotricha* sp. and *Cladophialophora* sp. treatments with a percentage increase of 135.27% and 85.19% against the control. The highest total dry weight value was obtained from the *Aspergillus* sp. treatment with a percentage increase of 46.34%. The shoot-root ratio values ranged from 1.13-1.43, indicating that the seeds already have roots supporting plant growth. The Dickson quality index ranged from 0.65-1.03, suggesting that the seedlings have good biomass distribution.

The fungi treatment did not significantly affect stem diameter growth because the seedling was still young. The endophytic fungi have the potential to increase the growth rate of sengon seedlings. For further research, the observation time needs to be improved. In addition, parameters for pest and disease resistance need to be evaluated.

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