Pathogenicity test of sengon (*Falcataria moluccana*) seed-borne endophytic fungus

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**Abstract.** The sengon (*Falcataria moluccana*) is one of the high-economic and highly cultivated industrial plants in Indonesia. Attacks by pests and diseases can affect quality and quantity and thus require effective control. One of the rare sengon plant fungus explorations has been developed. This study aims to find out the pathogenicity of 13 seed-borne endophytic fungi from sengon seeds. The result shows that seven endophytic fungi were pathogenic (*Daldinia sp, Curvularia lunata, Sardiomyces sp. Eremothecium ashbyi, Annulohypoxylon stygium. Arthrinium malaysianum, Annulohypoxylon nitens*) and six non-pathogenic (*Onchroconis humicola, Cladophialophora boppii, Aspergillus ruber, Aspergillus chevalieri, Ascotricha sp., and not identified fungi*). Thus the isolated endophytic fungi mainly were potentially pathogenic. Among the six species of non-pathogenic endophytic fungi, *O. humicola* is better able to increase the increase of plumules, radicals, cotyledons, and leaves higher than six other isolates.

1. **Introduction**

Sengon (*Falcataria moluccana*) is one of the most widely cultivated industrial plants in Indonesia. With a high economic value, appropriate cultivation is needed so that the quality of the wood remains maximal. However, pests and diseases are threats in the production of sengon wood as a forestry plant [1,2]. Therefore, several controls need to be pursued, one of which uses endophytic fungi from the plant itself. Exploration of endophytic fungi in these various plants shows the potential for endophytic fungi. However, there has not been much exploration of the potential of endophytic fungi in forestry plants, one of which is sengon.

Endophytic fungi are functional microbes found in plants and do not cause disease and live in healthy plants [3]. Endophytic fungi are polyphyletic fungi that generally belong to the ascomycetes group and grow in plant tissues of various types and habitats [4]. In general, endophytic fungi are in symbiosis with their host plants. The biological activity of secondary metabolites in endophytic fungi affects various aspects of agriculture, pharmaceuticals, and biotechnology in general.

Various plants have been widely studied related to the presence of endophytic fungi. In the research of Irawati et al.[5], the exploration of endophytic fungi on chili plants showed that 74.19% of isolates stimulated vegetative growth of seeds, red chili with 34 isolates were consistently non-pathogenic, and
12 isolates were pathogenic based on pathogenicity tests. In Handayani’s research [6], about 4-12% of the fungal isolates obtained were non-pathogenic and had the potential to be endophytic fungi that were antibiosis to *C. capsici* in multiple culture tests and endophytic phytate culture tests. These isolates have the potential as plant growth-promoting fungi (PGPF) and induce the formation of plant peroxidases. Various plants have been widely studied related to the presence of endophytic fungi. In the research of Irawati *et al.*[5], the exploration of endophytic fungi on chili plants showed that 74.19% of isolates stimulated vegetative growth of seeds, red chili with 34 isolates were consistently non-pathogenic, and 12 isolates were pathogenic based on pathogenicity tests. In Handayani's research [6], about 4-12% of the fungal isolates obtained were non-pathogenic and had the potential to be endophytic fungi that were antibiosis to *C. capsici* in multiple culture tests and endophytic phytate culture tests. These isolates have the potential as plant growth-promoting fungi (PGPF) and induce the formation of plant peroxidases.

In Sucipto's research [7], several isolates of bacteria and endophytic fungi obtained from rice plants showed high antibiosis activity in inhibiting *P. oryzae*. Endophytic isolates could reduce disease severity by about 30-70% in vivo tests. According to Hermawan [8], endophytic fungi from the death plant that were applied to sengon seeds could accelerate the growth rate and length of germination. Therefore, exploration of sengon plants needs to be done to determine the potential for endophytic fungi. Pathogenicity test in the exploration process of endophytic fungi is important to determine the character of the isolates obtained.

2. Method

2.1. Pathogenicity Test

Endophytic fungi used to result from isolation in sengon seeds, namely *Daldinia sp, Onchroconis humicola, Cladophialophora boppii, Curvularia lunata, Aspergillus ruber, Aspergillus chevalieri, Ascotricha sp, Sardiomyces sp, Eremothecium ashbyi, Annulohypoxylon stygium, Arthrinium malaysianum, and Annulohypoxylon nitens*. Endophytic fungi isolate grown on a Petri dish with potato dextrose agar (PDA) media.

Pathogenicity tests are performed by planting sengon seeds on a Petri dish containing PDA media that 2-week-old endophytic fungi have overgrown. Before planting, seeds are disinfected with heat treatment at 55 °C for 20 minutes and Natrium hypochlorite treatment 1% for 1 minute, then rinsed twice using sterile water. In one petri dish are planted 20 sengon seeds are planted and repeated three times for each endophytic fungi isolate. After two weeks of observed germinating power, plumule length, radicle length, number of leaves, and seed cotyledon.

2.2 Data Analysis

The data on the effect of fungi on the germination variable of sengon seeds were processed statistically and descriptively in the form of the average shown in tables and figures. Statistical analysis was performed using Analysis of Variance (ANOVA) with SNK data transformation test at 5% significance level using the SAS software version 9.0.

3. Results and Discussion

Pathogenicity tests on PDA media show potentially pathogenic (7 isolates) and potentially non-pathogenic isolates (6 isolates). Pathogenic fungi cause seeds to germinate abnormally or not even to germinate. Meanwhile, non-pathogenic fungi stimulated normal germination, characterized by optimum plumules (stems), radicles (roots), number of leaves, and cotyledons. The normality criteria for germination of sengon are indicated by the length of sprouts that reach twice the length of the seed and have a good growth shaft. Seeds that do not germinate are seeds that do not show growth until the end of observation.
Table 1. Percentage of seed germination conditions of endophytic fungus isolates.

| Treatment               | Normal | Germination (%) | Abnormal | Not Growing | Pathogenicity test |
|-------------------------|--------|-----------------|----------|-------------|--------------------|
| Control                 | 81.67<sup>a</sup> | 1.67<sup>d</sup> | 11.67<sup>b</sup> |            |                    |
| Daldinia sp             | 15.00<sup>cd</sup> | 48.33<sup>ab</sup> | 36.67<sup>a</sup> |            | Pathogen           |
| O. humicola            | 68.33<sup>ab</sup> | 0.00<sup>d</sup> | 31.67<sup>ab</sup> |            | Non Pathogen       |
| C. boppii              | 61.67<sup>ab</sup> | 6.67<sup>cd</sup> | 31.67<sup>ab</sup> |            | Non Pathogen       |
| C. lunata              | 43.33<sup>abc</sup> | 23.33<sup>bcd</sup> | 33.33<sup>ab</sup> |            |                    |
| A. ruber               | 56.67<sup>ab</sup> | 1.67<sup>d</sup> | 41.67<sup>a</sup> |            | Non Pathogen       |
| A. chevalieri          | 60.00<sup>ab</sup> | 1.67<sup>d</sup> | 38.33<sup>ab</sup> |            | Non Pathogen       |
| Ascotricha sp.         | 65.00<sup>ab</sup> | 1.67<sup>d</sup> | 33.33<sup>ab</sup> |            | Non Pathogen       |
| Sardiomyces sp         | 40.00<sup>bc</sup> | 3.33<sup>d</sup> | 56.67<sup>a</sup> |            | Pathogen           |
| E. ashbyi              | 23.33<sup>bc</sup> | 35.00<sup>abc</sup> | 40.00<sup>a</sup> |            | Pathogen           |
| A. stygium             | 10.00<sup>cd</sup> | 46.67<sup>ab</sup> | 43.33<sup>a</sup> |            | Pathogen           |
| Not identified fungi   | 78.33<sup>a</sup> | 1.67<sup>d</sup> | 20.00<sup>ab</sup> |            | Non Pathogen       |
| A. malaysianum         | 40.00<sup>bc</sup> | 16.67<sup>cd</sup> | 43.33<sup>a</sup> |            | Pathogen           |
| A. nitens              | 0.00<sup>d</sup> | 71.67<sup>a</sup> | 28.33<sup>ab</sup> |            | Pathogen           |

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

Quality germination has a good vigor that can become normal germination. In Table 1, the normal germination of sengon seeds grown on fungal isolates showed varying percentages compared to controls. Treatment of Daldinia sp, E. ashbyi, A. stygium, and A. nitens was significantly different from the control. Meanwhile, isolates O. humicola, C. boppii, C. lunata, A. ruber, A. chevalieri, Ascotricha sp, Sardiomyces sp, not identified fungi (SB11), and A. malaysianum were not significantly different from the control. The highest percentage of normal germination in SB 11 isolates was 78.33%, while the A. nitens isolates did not experience normal germination (0%). Based on the percentage of germination of these seeds, the fungus that gives the effect of normal germination to sengon seeds has the potential as an endophytic fungus. Endophytic fungi infect plants without causing symptoms, and some of them can also increase the growth of host plants [9]. Meanwhile, the treatment with a large percentage of abnormal sprouts indicated a potential pathogenic fungus. Four indicators of seed germination, such as plumule length, radicle length, number of leaves, and seed cotyledon length were compared with agronomic germination of control seeds (Table 2).

The agronomic differences in the seeds of each isolate varied. O. humicola isolate experienced plumular growth, which was not significantly different from the control, while the A. nitens isolate was significantly different from the control. The SB 11 isolate showed the same value as the control in the length data, while the A. nitens isolate was significantly different from the control. The number of leaves formed on the seeds during observation, the control was significantly different for all isolates. The length of the seed cotyledons observed in the control treatment was not significantly different from the 12 isolates but was significantly different from the Sardiomyces sp isolate.
Table 2. Agronomic data on seed germination of endophytic fungi isolates.

| Treatment         | Plumule length | Radicular length | Total of leave | Cotyledon length |
|-------------------|----------------|------------------|----------------|------------------|
| Control           | 2.90<sup>ab</sup> | 0.88<sup>a</sup> | 0.25<sup>c</sup> | 0.807<sup>a</sup> |
| Daldinia sp       | 0.94<sup>bc</sup> | 0.13<sup>d</sup> | 0.10<sup>cd</sup> | 0.583<sup>ab</sup> |
| O. humicola      | 3.85<sup>a</sup>  | 0.62<sup>ab</sup> | 1.47<sup>a</sup>  | 0.645<sup>ab</sup> |
| C. boppii        | 2.67<sup>ab</sup> | 0.77<sup>ab</sup> | 0.00<sup>d</sup>  | 0.615<sup>ab</sup> |
| C. lunata        | 0.78<sup>bc</sup> | 0.29<sup>cd</sup> | 0.07<sup>d</sup>  | 0.588<sup>ab</sup> |
| A. ruber         | 2.10<sup>abc</sup>| 0.67<sup>ab</sup> | 0.10<sup>cd</sup> | 0.553<sup>ab</sup> |
| A. chevalieri    | 2.52<sup>ab</sup>| 0.75<sup>ab</sup> | 0.13<sup>cd</sup> | 0.625<sup>ab</sup> |
| Ascotricha sp.   | 2.95<sup>ab</sup>| 0.53<sup>abc</sup>| 0.27<sup>bcd</sup>| 0.630<sup>ab</sup> |
| Sardiomycetes sp.| 1.92<sup>abc</sup>| 0.41<sup>bc</sup> | 0.47<sup>bc</sup> | 0.385<sup>b</sup>  |
| E. ashbyi,       | 1.08<sup>abc</sup>| 0.49<sup>abc</sup> | 0.90<sup>ab</sup> | 0.587<sup>ab</sup> |
| A. stygium       | 0.64<sup>bc</sup> | 0.12<sup>d</sup>  | 0.15<sup>cd</sup> | 0.460<sup>ab</sup> |
| Not identified fungi | 3.08<sup>ab</sup> | 0.78<sup>a</sup>  | 0.13<sup>cd</sup> | 0.708<sup>ab</sup> |
| A. malaysianum   | 1.59<sup>abc</sup>| 0.27<sup>cd</sup> | 0.10<sup>cd</sup> | 0.490<sup>ab</sup> |
| A. nitens        | 0.38<sup>c</sup>  | 0.05<sup>c</sup>  | 0.00<sup>d</sup>  | 0.630<sup>ab</sup> |

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

Each endophytic potential fungal isolate showed a different effect so that the agronomic seeds varied compared to the control. Some isolates stimulated seed growth in certain parts, such as root boosters, stem boosters, or leaf growth promoters. The seeds infected with seed-borne pathogens then treated with endophytic fungus supernatants showed a faster germination rate with a higher percentage of germination than seeds to controls [10]. The length of the coleoptile (stem future) and radicle (root shoot) in the treatment of seeds infected with the plague fungus treated with endophytic fungus supernatant was higher than the control. In addition, endophytic fungi can increase plant resistance to insects, nematodes, and other pathogens [9,10]. According to the data in Table 2, endophytic fungi O. humicola stimulated stem growth faster than other isolates.

4. Conclusion
The result shows that seven endophytic fungi were pathogenic (Daldinia sp, Curvularia lunata, Sardiomycetes sp, Eremothecium ashbyi, Annulohypoxylon stygium, Arthrinium malaysianum, Annulohypoxylon nitens) and six non-pathogenic (Onchroconis humicola, Cladophialophora boppii, Aspergillus ruber, Aspergillus chevalieri, Ascotricha sp., and not identified fungi). Thus the isolated endophytic fungi mainly were potentially pathogenic. Among the six species of non-pathogenic endophytic fungi, O. humicola is better able to increase the increase of plumules, radicals, cotyledons, and leaves higher than six other isolates.

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