Fungi associated with *Falcataria moluccana* (L.) seed

Y Istikorini*, A S Wulandari¹, and O Y Sari¹

¹Department of Silviculture, Faculty of Forestry, IPB University, Bogor 16680, West Java, Indonesia

*Email: yunik.istikorini@gmail.com

**Abstract.** Seeds are the most important input of any tree cultivation. Qualified seed is defined as one pure variety with high germination rate and free from pathogens. Qualified seed ensures good germination, rapid emergence, and vigorous growth. Sengon (*Falcataria moluccana*) is one of the major forest trees in Indonesia, particularly in West Java. This study aimed to detect and identify fungi of *F. moluccana* seeds and to determine the pathogenicity of the isolates. The method of fungi isolation was blotter test with 7-8 d of incubation period. As much as 7 fungi isolates were obtained based on the method. The isolates were *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia* sp., *Chaetomium globosum*, *Penicillium* sp. and S5 isolate. *A. flavus*, *A. niger* and *Penicillium* sp. were found on sengon seeds in both treatments, with and without surface sterilization. Pathogenicity test showed that fungi within or on *F. moluccana* seeds had potential to be pathogens on seeds.

1. Introduction
The availability of seedlings is important for forest management program. Seedling stock depends on healthy seedling conditions [1]. It could be affected by biotic and abiotic factors. One of the biotic factors is pathogen. Thus, it is important to obtain healthy seeds to reduce the possibility of pathogen infection on tree.

Healthy seeds are defined as seeds that could germinate well and free from pathogen. Previous research showed that pathogens could live in plant seeds with 3 ways [2]. First, pathogens are laid inside the seed tissue. Second, pathogens are contaminated the seed surface. Third, pathogens are mixed with seeds. These conditions could drive pathogens to attack the seeds or other plants in the surrounding area.

In forest tree, there are several reports on seed-borne pathogens. Various pathogens were found on *Acacia mangium*, *Acacia crassicarpa* and *Acacia auriculiformis* [3]. Several studies has also explored pathogens on *Calophyllum inophyllum*, *Swietenia macrophylla*, and *Hevea brasiliensis* seed [4-6]. There are no many studies that worked on potential seed-borne fungi.

At recent time in forest industry, *F. moluccana* has been cultivated in many areas in Indonesia. Sengon is a fast-growing species, therefore it becomes one of the major forest trees in Indonesia, particularly in West Java. The development of this industry has generated homogenous forests. Hence, It would rise the vulnerability of forest tree towards pathogens attack. Unfortunately, there are no many awareness toward seed health, although pathogen control since pre-harvest to post-harvest is more effective than just post-harvest control [7].

Seed quality needs to be assured as it is correlated to seed health, especially for the seed suppliers. Seed-health testing is one of the factors that should be developed to enhance the productivity in forest management activity. Therefore, this study aimed to detect and identify fungi associated with *F. moluccana* seeds and to determine the pathogenicity of the isolates.
2. Methods

2.1. Time and location
This study was conducted in Laboratory of Forest Pathology, Department of Silviculture, Faculty of Forestry, IPB University, from January to April 2019. Sample of sengon seed was obtained from the Permanent Seed Nursery, Faculty of Forestry, IPB University.

2.2. Germination test
Germination test on sengon seed was began with breaking the seed dormancy. Sengon seeds were put into hot water for 5 min, then into distilled water for 12 hr. Surface sterilization on sengon seeds was applied by soaking the seeds in sodium hypochlorite 1% for 3 min, then rinsed 3 times with distilled water. Control treatment was applied without surface sterilization. Each treatment used 100 seeds (20 seeds/petri dish) and incubated for 7-8 d at room temperature. The germination rate was observed.

2.3. Blotter test
The method referred to previous study with several modifications [8]. F. moluccana seeds were soaked in sodium hypochlorite 1% for 3 min, then rinsed 3 times with distilled water. Control treatment was applied without surface sterilization. Sengon seeds were placed in the double sterile filter paper that already moistened with distilled water. Each treatment used 100 seeds (20 seeds/petri dish) and then incubated for 7-8 d at room temperature. Germination rate and disease incidence were then observed.

2.4. Fungi isolation, purification, and morphological identification
The seeds that covered with mycelium were isolated in petri dish containing potato dextrose agar (PDA). After incubation at room temperature for 7-10 d, the isolates were purified by put it onto potato dextrose agar and water agar (WA) medium. The isolates identified macroscopically and microscopically based on the morphological characters of the colonies.

2.5. Pathogenicity test of seed-borne fungi
Sengon seeds were soaked in sodium hypochlorite 1% for 3 min and then rinsed in distilled water 3 times. Sengon seeds were inoculated with 7 d old fungi isolates. Each treatment used 50 seeds (10 seeds/petri dish). Control treatment was applied on potato dextrose agar medium without isolate culture. The treatments incubated at room temperature for 14 d. Then, disease incidence was observed.

3. Results and discussion

3.1. Germination rate of F. moluccana seed
Sengon seeds showed higher germination rate on treatments with broken dormancy combination than the others (Table 1). The highest germination rate was combination treatment of broken dormancy and without surface sterilization as much as 88% and the lowest was without surface sterilization treatment as much as 33%. Based on Duncan Multiple Rate Test, the treatments with broken dormancy combination were significantly different with single treatments. Sengon seed is one of the seeds that has physical dormancy, thus water could not permeate through the seed coat [9]. Therefore, breaking the dormancy of the seed is needed in order to germinate well.

| Treatments                             | Germination rate (%) |
|---------------------------------------|----------------------|
| No surface sterilization              | 33\textsuperscript{a} |
| Surface sterilization                 | 34\textsuperscript{b} |
| Broken dormancy-no surface sterilization | 88\textsuperscript{a} |
| Broken dormancy-surfaces sterilization | 77\textsuperscript{a} |

\textsuperscript{a}Numbers followed by the same letter are not significantly different on 5% Duncan Multiple Rate Test.
3.2. Fungi associated with F. moluccana seeds

Over the several isolates that could be obtained, there were 7 isolates that had different morphological character. The isolates were collected from with and without surface sterilization treatments. S1 isolate was identified as Aspergillus niger. The upper surface side of the isolate culture formed black to dark brown colonies and the reverse side formed white to light yellow colonies. The conidia was black to dark brown with biseriate conidiophores, spherical vesicles, hyaline or lightly pigmented hyphae near the apex, round and wrinkle conidia. The second isolate or S2, was identified as Aspergillus flavus. The upper surface side of the isolate culture formed rough pale green to brown colonies and the reverse side formed golden brown colonies. The conidia was pale green to brown with biseriate conidiophores, rather spherical vesicle with ¾ metula covering, hyaline or lightly pigmented hyphae near the apex, globose ellipsoid and smooth to finely roughened conidia.

S3 isolate was identified as Penicillium sp. The upper surface side of the isolate formed greenish blue colonies and the reverse side formed yellowish white colonies. It has branched conidiophores, brush-like spore head, ampulliform phialides, spherical conidia and chains of single-celled spores. Meanwhile, S4 isolate was identified as Chaetomium globosum. The upper surface side of the isolate formed white at the center with grayish dark green spot colonies and the reverse side formed more yellowish colonies. It has globose, ellipsoid or ovate ascomata, formed long chain without branches, brown lemon-shaped ascospores and smooth-surface with apical papillae.

S5 isolate was unidentified. The upper surface side of the isolate formed white fluffy colonies and the reverse side formed yellowish white colonies. It has hyaline hyphae, non-septate hyphae and formed flowery hyphae at the end of hyphae branch. S6 isolate was identified as Botryodiplodia sp. The isolate produced white thin mycelium then turned into grey and finally into blackish colonies. It has hyaline hyphae, non-septate hyphae, conidia were subovoid to ellipsoidal in shape and the hyaline conidia then turned into brown double layer conidia. The last isolate, S7, was identified as Alternaria sp. The isolate produced a very thin mycelium formed white colonies. It has hyaline hyphae, septate hyphae, short geniculate conidiophores, obovoid conidia, single or in chains conidia, and might be formed secondary conidiophores at the apex.

3.3. Fungi species isolated from F. moluccana seed with and without surface sterilization

Species of fungi that associated with sengon seeds based on the treatments of with and without surface sterilization were shown in Table 2. A. flavus, A. niger and Penicillium sp. were found in both treatments. C. globosum and S5 isolates were only found in no surface sterilization treatment while Alternaria sp. and Botryodiplodia sp. were only found in surface sterilization treatment.

Table 2. Species of fungi isolated from Falcataria moluccana seeds

| Fungi Type       | No Surface Sterilization | Surface Sterilization |
|------------------|--------------------------|-----------------------|
| Aspergillus flavus | Alternaria sp.           |                       |
| Aspergillus niger | Aspergillus flavus       |                       |
| Chaetomium globosum | Aspergillus niger     |                       |
| Penicillium sp.   | Botryodiplodia sp.      |                       |
| S5 isolate       | Penicillium sp.         |                       |

Alternaria, Aspergillus, Chaetomium and Penicillium are common storage fungi and generally found associated with seeds that were stored for a certain period. The fungi primarily invade the embryo. At early stage of infection, the seed may appear normal but it could interfere the embryo growth, thus the seed could not germinate well. Storage fungi might be present as dormant spores or mycelium on the seed surface or below the pericarp. They would active and multiply under favourable storage conditions [10].

The inoculum of Aspergillus and Penicillium might invade the seed embryo, thus they were found in both treatments. In addition, the light spores and rapid growth of the fungi make them grew well though the seed surface had been sterilized. Meanwhile, C. globosum were also categorized as a soft-rot fungi that has low wood-weathering ability [11]. The inoculum of C. globosum might be stuck on the seed surface due to harvest activity, thus it was found in no surface sterilization treatment only. In
the same case, the inoculum of *Alternaria* sp. might also present below the pericarp because it was found only in surface sterilization treatment.

*Botryodiplodia* sp. was categorized as a weak parasite fungus which infection was carried out through lesion [12]. The inoculum could be found inside several plant parts such as seeds, seed coat, branch and twig [13]. The fungi was assumed infested within the sengon seed, because it was not found in surface sterilization treatment. The fungi was then able to attack the plant in suitable conditions.

3.4. Pathogenicity test of fungi associated with *F. moluccana* seeds

The fungi caused the highest disease incidence were *Alternaria* sp., *A. flavus* and *A. niger* with 100% disease incidence, respectively. *Penicillium* sp., *Botryodiplodia* sp. and S5 isolates had relatively high pathogenicity with 80-96% of disease incidence. *C. globosum* had the lowest pathogenicity with 78% of disease incidence. Based on the data, the fungi were considered as potential pathogens as it caused disease symptoms on sengon seed with a rather high percentage (Table 3).

| Fungi Type        | Seeds with the Disease Symptom<sup>a</sup> (%) | Disease Incidence (%) |
|------------------|-----------------------------------------------|-----------------------|
| *Alternaria* sp.  | NG  66   FG  30  IG  4  GW  0                | 100                   |
| *Aspergillus flavus* | NG  72   FG  18  IG  10  GW  0             | 100                   |
| *Aspergillus niger* | NG  98   FG  2   IG  0  GW  0             | 100                   |
| *Botryodiplodia* sp. | NG  54   FG  2   IG  24  GW  20         | 80                    |
| *Chaetomium globosum* | NG  58   FG  8   IG  12  GW  22         | 78                    |
| *Penicillium* sp.  | NG  56   FG  0   IG  40  GW  4           | 96                    |
| S5 isolate        | NG  72   FG  0   IG  8  GW  20           | 80                    |

<sup>a</sup> Note: NG = not germinated, FG = failed to germinate, IG = infected germination, and GW = well germinated.

*Alternaria* sp., *A. flavus* and *A. niger* had caused 100% of disease incidence. The symptoms were similar. The seeds were covered with colony of fungi, thus most of the seeds were rotten and could not germinate. *A. niger* did not provide the seed to germinate, while *Alternaria* sp. and *A. flavus* still provide 4%-10% ability for the infected seed to germinate. The mechanism of the infection was assumed through mycotoxin [14], hence it caused 100% disease incidence.

The symptoms of *Botryodiplodia* sp. associated with *F. moluccana* seed were seed coat covered by colony of fungi, thus resulted a rotten seed, gummosis, and root necrosis. As much as 20% of seeds could germinate well because the fungi colony which covering the seed coat were not infecting the sengon sprout. The attack of *Botryodiplodia* sp. caused root rot, damping-off, wilt, gummosis and generally would decrease the germination rate, as well as death on *Pinus* spp. and *Mangifera indica* [15].

The symptoms of *C. globosum* were similar to *Botryodiplodia* sp. The seed coat were covered with fungi colony, thus the seeds could not germinate well, then became rotten and gummosis. As much as 22% of seeds could germinate well. The ascospores of *C. globosum* were grown rapidly and covered the seed coat with thick mycelium. The mechanism was originally used to suppress the attack of soil-borne pathogens [16]. The attack severity was assessed lower than soil-borne pathogens. It allowed the seed to survive and keep growing well.

*F. moluccana* seeds infected by *Penicillium* sp. had similar symptoms with *A. flavus*. Most of the seeds were rotten and could not germinate well because the seeds were covered with colony of fungi. The sprout which could germinate indicated growth inhibition due to the growth of mycelium and spores on seed surface. The attack of *Penicillium* sp. on seed caused inhibition on seed germination, radicle and coleoptile elongation [17].

S5 isolate caused a rather high disease incidence as much as 80%. The symptoms were similar to the others, root necrosis and seed covered by mycelium.
4. Conclusion
There are 7 fungi associated with *F. moluccana* seeds. Those fungi are *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia* sp., *Chaetomium globosum*, *Penicillium* sp. and S5 isolate. Pathogenicity test shows that all of them have potential to be phytopathogen.

References
[1] Budi S W 2006 Modul Pelatihan Penanaman Pohon ITTO Training Proceedings, Muara Bulian 4th-6th May 2006 pp 25-33
[2] Sutakaria Y 1988 *Diktat Penyakit Benih* (Bogor: IPB University)
[3] Suharti T, Joko T and Arwiyanto T 2017 *J. HPT Tropika*. 17(1) 19-36
[4] Yuniarti N, Suharti T and Rustam E 2015 *Pros. Sem. Nas. Masy. Biodiv. Indon.* 1(6) 1442-1447
[5] Putri K P, Bramasto Y and Suharti T 2011 *Tekno Hutan Tanaman* 4(1) 1-6
[6] Novariza D A, Lubis L, Sitepu S F and Tistama R 2015 *Jurnal Agroekoteknologi* 4(1) 1925-1936
[7] Rahayu M 2016 *Buletin Palawija* 14(1) 78-88
[8] Sutopo L 1993 *Teknologi Benih* (Jakarta: PT Raja Grafindo Persada)
[9] Mulawarman, Roshetko J, Sasongko S M and Iriantono D 2002 *Pengelolaan Benih Pohon: Sumber Benih, Pengumpulan, dan Penanganan Benih* (Bogor: International Centre for Research in Agroforestry and Winrock International)
[10] Arya A and Perello A E 2010 *Management of Fungal Plant Pathogens* (UK: CAB International)
[11] Djarwanto, Suprapti S and Hutapea F J 2018 *Jurnal Penelitian Hasil Hutan* 36(2) 129-138
[12] Semangun H 2007 *Penyakit-penyakit Tanaman Hortikultura di Indonesia Edisi ke-2.* (Yogyakarta: Gajah Mada University Press)
[13] Salamiah 2008 *Agrin.* 12(1) 86-99
[14] Sawane A and Sawane M 2014 *International Journal of Current Microbiology and Applied Sciences* 3(11) 116-121
[15] Maciel C G, Muniz M F B, Mezzomo R and Reiniger L R S 2015 *Scientia Forestalis* 43(107) 639-646
[16] Hubbard J P, Eckenrode C J and Harman G 2011 *Canadian Journal of Microbiology* 28(4) 431-437
[17] Rao V K, Girisham S and Reddy S M 2014 *J. Biochem. Tech.* 5(4) 382-387