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Endophytic Fungi in *Paraserianthes falcataria:* Production of Indole Acetic Acid

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Identification of endophytic fungi in *Paraserianthes falcatria* is the effort of obtaining the potential of endophytic fungi as phytohormone producer. Phytohormone is needed to spur shoot and root initiation. This study in *P. falcatariais* is necessary, because woody of *P. falcataria* decreases every year. The aims of the study were to identify endophytic fungi from leaves, twigs, and roots of *P. falcataria*, and determine Indole Acetic Acid (IAA) content from endophytic fungi. Isolates that were grown from leaves, twigs and roots cuttings on PDA, were identified based on micro- and macromorphology. Determining of IAA content was conducted with spectrophotometer *vis* based on a calibration curve from the standard solution. Ten of isolates fungi from leaves, twigs, and roots was obtained. However, only nine isolates that could be identified. They were *Aspergillus* sp., *Acremonium* sp., *Cladosporium* sp., *Trichoderma* sp. 1, *Phytium* sp., *Rhizoctonia* sp., *Trichoderma* sp. 2, *Hormiscium* sp. 1 and *Hormiscium* sp. 2. Production of indole acetic acid (IAA) from *Cladosporium* sp. had the highest content than others (311 ppm). The lowest IAA content (51.97 ppm) was produced by the *Rhizoctonia* sp. The study can be continued to know their ability as Plant Growth Promoting Fungus (PGPF) agents and biopesticides of *P. falcataria* seedlings.

Key words: endophytic fungi, identification morphologically, *Paraserianthes falcataria*

Identifikasi jenis jamur endofit yang bersimbiosis pada *Paraserianthes falcatria* sebagai tanaman inang merupakan bagian dari upaya menggali potensi jamur endofit sebagai penghasil senyawa fitohormon. Fitohormon diperlukan untuk memacu pembentukan tunas dan inisiasi akar pada kultur jaringan tanaman. Studi ini sangat penting mengingat *P. falcataria* merupakan tanaman hutan yang produksi kayunya menurun tiap tahunnya. Tujuan penelitian ini adalah untuk mengidentifikasi jamur endofit yang diisolasi dari daun, akar dan ranting tanaman *P. falcataria* dan mengukur kandungan *Indole Acetic Acid* (IAA) yang diproduksi oleh jamur endofit. Isolat yang tumbuh dari potongan daun, ranting dan akar pada media PDA diidentifikasi berdasarkan morfologi mikro dan makro. Penentuan kandungan IAA diukur menggunakan spektrofotometer *vis* berdasarkan kurva kalibrasi yang dibentuk oleh larutan standar. Hasil penelitian diperoleh 11 isolat jamur endofit yang berasal dari daun, akar dan ranting tanaman *P. falcataria*. Namun hanya sembilan isolat yang teridentifikasi sampai tingkat genus adalah *Aspergillus* sp., *Acremonium* sp., *Cladosporium* sp., *Trichoderma* sp. 1, *Phytium* sp., *Rhizoctonia* sp., *Trichoderma* sp. 2, *Hormiscium* sp. 1 dan *Hormiscium* sp. 2. Produksi IAA dari *Cladosporium* sp. memiliki kandungan IAA tertinggi (311 ppm) dibandingkan isolat lainnya. Kandungan IAA terendah (51,97 ppm) dihasilkan oleh *Rhizoctonia* sp. Hasil penelitian ini dapat dilanjutkan untuk mengetahui kemampuannya sebagai agen *Plant Growth Promoting Fungus* (PGPF) dan biopestisida bibit *P. falcataria*.

Kata kunci: fungi endofitik, identifikasi morfologi, *Paraserianthes falcataria*

Paraserianthes falcataria is the type of woody plant that has economic value. Economically, the demand of *P. falcataria* increases every year. Meanwhile, The Minister of Forestry stated that the demand for *P. falcataria* wood reached 2 million m^3 in 2010, and 3 million m^3 in 2011 (Daryanto 2010). This demand can be fulfilled through the planting of *P. falcataria* in the social forest (in the agroforestry or tree on the farm). It was proven that the production values of *P. falcataria* in 2014 increased about 7.22% from 2.788.455,18 m^3 in 2013 (Central Statistics Agency (BPS) 2014). Another benefit of *P. falcataria* is that it can repair the marginal land, through the presence of root nodules. Root nodules are the symbiosis between the roots with nitrogen-fixing bacteria (*Rhizobium*) that can increase soil fertility.

The growth of *P. falcataria* can not be separated from the presence of endophytic fungi (in addition to *Rhizobium*) in *P. falcataria* roots. Endophytic fungi are micro fungi that colonize plant tissue without produce symptoms or other negative effects on host plants (Stone *et al.* 2000). The fungi synergize with a host plant in the form of mutualism symbiosis. Endophytic fungi can increase plant health, both directly and indirectly. Directly, endophytic fungi produce special substances such as metabolite secondary which can inhibit the infection of the pathogen and pest (Debbab *et al.* 2009). Indirectly, they produce the phytohormone compound that can

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increase a host resistance to the extreme environment and pest-diseases (Denance *et al.* 2013). Some of the phytohormone compound that was produced by endophytic fungi, were IAA, gibberellin, dan cytokinin (Lu *et al.* 2000; Barka *et al.* 2002). IAA plays an important role in the growth cells and division, tissue differentiation and responds to light and gravity (Lebuhn and Hartmann 1993). IAA can also spur the growth of tissue culture. This was proven when the formation of callus occurs after inoculation of *Colletotrichum* sp. in tissue culture of *Althennnantera sesillis* (Subbarayan *et al.* 2010).

The study related to the influence of phytohormoneon plants, showed that not all endophytic fungi can increase the growth plant (Dai *et al.* 2008). Some of the phytohormone compounds such as abscisic acid (ABA) can inhibit the growth of the host plant. Endophytic fungi like *Fusarium* sp. is able to produce more phytohormone compounds. *Fusarium* sp. from *E. pekinensis,* produce IAA and gibberellin but gibberellin does not influence the increasing plant growth (Dai *et al.* 2008).

Related to the ability of endophytic fungi to produce phytohormone compound, the conservation effort of the forest plant species such as *P. falcataria* can be spur through introducing endophytic fungi in its tissue plant. As the first step, this research was aimed to study diversity and morphological identification of endophytic fungi from the plant, and determine the IAA content of these fungi.

MATERIALS AND METHODS

Identification Morphologically and Diversity of Endophytic Fungi from *Paraserianthes falcataria.* The plant's parts of *P. falcataria* that were used as the samples of fungi isolation, were leaves, twigs, and roots. Leaves, twigs and root samples were soaked in Tween 80 (2-3 drops of Tween 80 in 50 mL of sterile water), shaken with a shaking incubator at a speed of 120 rpm, 5 minutes at room temperature. Samples were rinsed with sterile water to remove residues. The samples surface was sterilized in 50 mL of perchloricacid (1%), shaken in shaking incubator at a speed of 120 rpm for 5 minutes. That every sample was rinsed again with sterile water, dried on filter paper, aseptically. Then, they were cut into 0.5-1 cm. They were placed on PDA which contained 80 µg mL of streptomycin and incubated at room temperature (27–28°C) for 4-7 days. A single colony that grew, was re-cultured on PDA by the tilted agar method as working culture and stock culture (Suciatmih *et al.* 2011). Fungi isolates were characterized morphologically using Munshell (1996), Introduction to Fungi by Webster and Weber (2007).

The Abundance and Diversity of Fungi. The abundance and diversity of fungi are the parameters that related each other. The abundance of fungi species is counted based on Pawtowska *et al.* (2014). The diversity of fungi were determined based on Shanon-Winner index (Zivanoch *et al.* 2017).

Phytohormone from Endophytic Fungi. Isolates were cultured on PDB added tryptophane (1%). Incubation of isolates using a shaker at a speed of 90 rpm for 13 d, in the dark condition. For quantitative evaluation, a calibration curve was initially made using 300 mg mL^{-1} IAA that diluted with distilled water to obtain increased concentration (0, 10, 20, 50, 100, 210, 430, and 500 ul mL^{-1}), then centrifugated at a speed of 8000 rpm, 4 °C for 10 min. One mL of supernatant was removed and transferred to a test tube, added 1 mL of Salkowski reagent (0.62 g $FeCl_3.6H_2O$; 33 mL H_2O; and 50 mL H_2SO_4). The test tube was established for 15 minutes, in dark condition, then IAA production was determined by Colorimetry spectrophotometry (530 nm) (Mehmood *et al.* 2018; de Melo Pereira *et al.* 2012).

RESULTS

The number of plants parts that were used for isolation of endophytic fungi, were 75 pieces. They consisted of 25 pieces of leaves, 25 pieces of twigs and 25 pieces of roots. From 75 pieces, only 10 pieces of leaves, 5 pieces of twigs and 6 pieces of roots that were colonized by endophytic fungi. The endophytic fungi consisted of 15 isolates from leaves (4 fungi genus), 7 isolates from twigs (4 fungi genus) and 7 isolates of roots (4 fungi genus). Based on the number of plant parts that were colonized by fungi, the leaves have a greater percentage of colonization (48%) than twigs. The number of colonization in twigs were relatively the same as the roots (Fig 1).

The results showed that genus of fungi that colonized in leaves, were *Aspergillus* sp., *Cladosporium* sp., *Trichoderma* sp. 1 and *Acremonium* sp. *Hormiscium* sp. 1, *Hormiscium* sp. 2, *Acremonium* sp. and *Aspergillus* sp., isolated from the twigs. *Trichoderma* sp. 2, *Rhizoctonia* sp., *Phytium* sp. and one unidentified isolate were founded to colonize in roots (Fig 2). Isolates of fungi were identified based on morphological characteristics both micro and macro

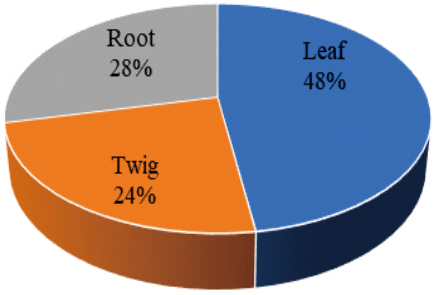
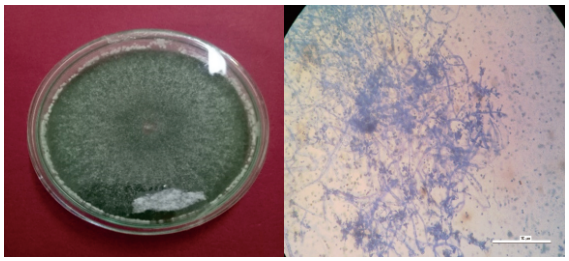
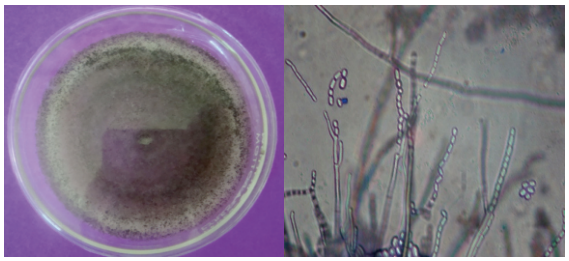


Fig 1 The colonization rate of endophytic fungi in *P. falcataria.*


Trichoderma sp.1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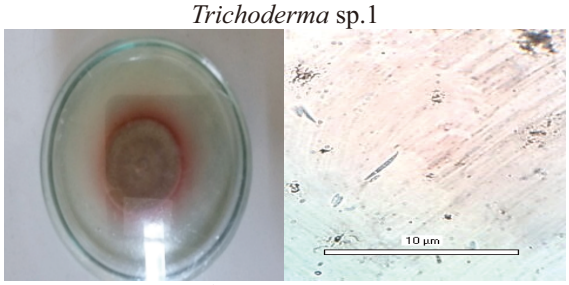
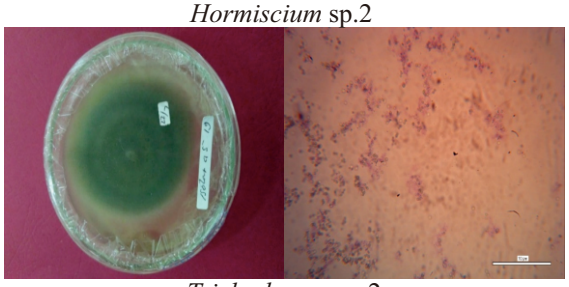
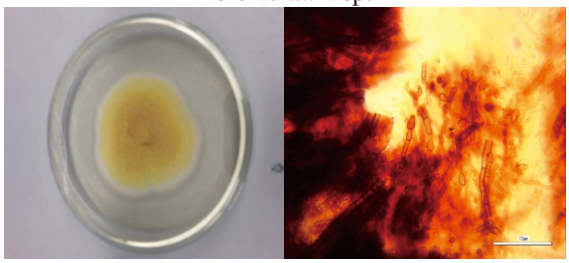
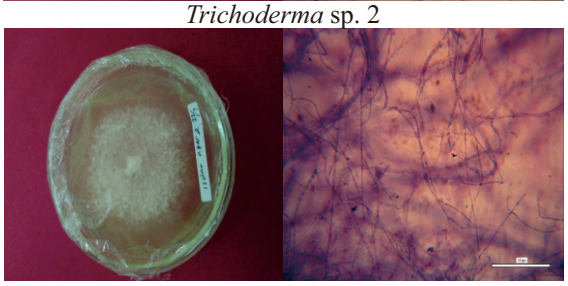
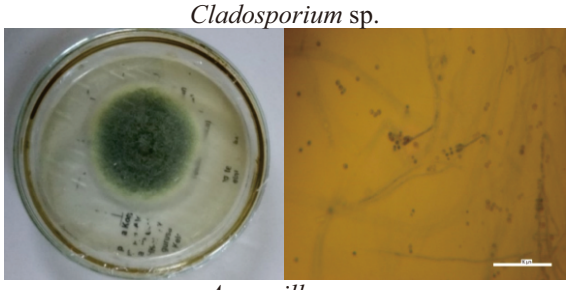


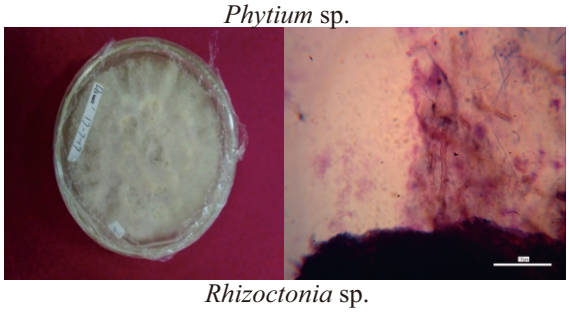
Acremonium sp.


Trichoderma sp. 2


Cladosporium sp.


Phytium sp.


Aspergillus sp.


Rhizoctonia sp.

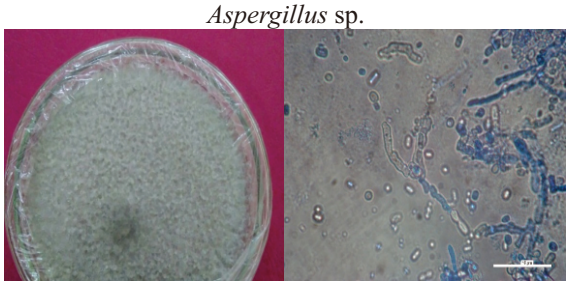

Hormiscium sp.1

Fig 2 The endophytic fungi isolates from *P. falcataria*.

(Table 1).

The difference inter genus of fungi was clear both macroscopically and microscopically, include *Cladosporium* and *Hormiscium.* Both of them have conidia arranged like branched chains and round-oval shaped. Two *Trichoderma* isolates (*Trichoderma* sp. 1 from leaves and *Trichoderma* sp. 2 from roots) and two *Hormiscium* isolates (*Hormiscium* sp. 1 and 2 from twigs) were different isolates. This was proven through the compatibility testing between isolates.

The plant parts that were isolated, showed that there was a difference in the number of individual species. Relative abundance of fungi species was different in leaves, twigs, and roots (Table 2). Fungi isolates that colonized leaves with 40% the relative abundance were *Trichoderma* sp. 1. In twigs, isolates that had 42.86% the relative abundance was *Hormiscium* sp. 1. *Trichoderma* sp. 2 was species that had the highest relative abundance (33.333%) in the roots. The high relative abundance showed that *Trichoderma* sp. 2 was able to compete and grow in the same niche, with the other fungi. *Trichoderma* was a genus that had the highest competitive ability than the other fungal groups. While *Trichoderma* did not colonize in the roots, another fungal genus (*Hormiscium*) became dominant among other fungi.

The abundance of species determines the species diversity in the community. Diversity of endophytic fungi from leaves, twigs and roots, was moderate (H' leaves = 1,3093; H' twigs = 1.277; H' roots = 1.3050) (Table 3). The Shannon-Wener index of fungi species from leaves, twigs, and roots were 0.9446, 0.9212 dan 0.9414 respectively (Table 3). This showed that evenness of fungi species in all of the *P. falcataria* parts that were isolated was high. The species diversity also explainedthe dominance of fungi species. The Dominance index of fungi species was close to 1 or 1. This indicated that the dominance pattern was centralized, whereas if the dominance index is close to 0, it indicates that no species dominate in its community (Odum, 1993). According to it, the results showed that there was no concentration of dominance to one endophytic fungi species (Table 3).

Production of IAA by Endophytic Fungi. Endophytic fungi are fungi that its infection does not cause physiological changes in the plant. One of the benefits of the presence of these fungi is the ability to produce phytohormone compounds, such as IAA. This can stimulate plant growth, maximally. Especially in micropropagation (tissue culture), the introduction of endophytic fungi is very important to increase IAA compound in the plant.

This result showed that each fungi species had different IAA production capability. *Cladosporium* sp. (isolate from leaves) had the highest absorbance value of 0.3198 and the lower absorbance value (0.0345) was produced by *Rhizoctonia* sp (Table 4).

DISCUSSION

Some research suggested that colonization in roots by endophytic fungi was the highest than other parts of the plant. Roots and rhizosphere are areas with a high level of competition between parasites and saprophytes. The roots will give positive or negative influence to the competition. However, in this research, the high colonization by endophytic fungi occurred in leaves (Fig. 1). Jia *et al.* (2016) explained that the colonization by endophytic fungi was determined by compound secreted from plant. Roots and leaves that area are rich nutrient (absorption from soil and production of photosynthesis), cause a high colonization of fungi. However, in colonization of fungi in root can decrease because of endosymbiont competition (Jia *et al.* 2016). Twig was an area with a low level of colonization, which indicated a low nutrient content than in leaves and roots.

The ability of endophytic fungi to colonize plant, is determined by species of fungi. This is related with the ability of competition to niche and nutrition. This is explained that the symbiosis form between endophytic fungi and plant host (parasitism and mutualism) is determined by the genotype host colonized (Unterseher and Schnittler 2010). The host plants largely determined the colonization and distribution of endophytic fungi in the host plants (Saikkonen *et al.* 2004).

The macromorphological character is not the specific characteristics to identify fungi to the species level (Suryantini *et al.* 2015). Isolate color usually was determined by age of fungi and environment/media condition. Isolate of *Aspergillus, Trichoderma,* and *Hormiscium* have a similar color (between green - dark green) although there was a difference of value color based on Mushell color (Munsell 1961). *Acremonium* has a similar color with *Fusarium,* namely between white - purplish. This was different from isolates of *Cladosporium.* They had a variety in color (Ogorek *et al.* 2012). *Cladosporium* sp. (in this research) was yellow colored (Fig. 2). *Rhizoctonia* isolates were white–dark brown. Suryantini *et al.* (2015) showed that binucleate *Rhizoctonia* isolates were white while

Table 1 Morphological characterization of endophytic fungi

Isolate from Segment of plant	Macroscopic characteristics			Microscopic characteristics	Species of fungi
	The color of isolate based on Munsell color chart	Concentric ring	Air hypha		
Leaf	10 GY 3/1	Yes	No	Hyphae are septate,strigma at the tip of hyphae, green conidia.	*Aspergillus* sp.
	7.5 R 3/6	Yes	No	Hyaline and multicellular conidia, 3-4 celled conidia, crescent-shaped.	*Acremonium* sp.
	2.5Y ¾	No	No	Brown-dark conidia thatare formed branching chains/single conidia.	*Cladosporium* sp.
	7.5GY 5/6	Yes	No	Hyphae are septate, branching out, formed phialides near the tip of hyphae, conidia are green and globose.	*Trichoderma* sp.1
Twig	5 GY 7/2	Yes	No	Conidia are hyaline-brown, form branching out.	*Aspergillus* sp.
	7.5 R 3/6	Yes	Yes	Conidia were hyaline, 2-3 celled, crescent-shaped.	*Acremonium* sp.
	5 GY 6/1	No	No	Conidia are a darkcolor, globose, branching out.	*Hormiscium* sp.1
	5 GY 4/2	No	No	Conidia are a darkcolor, globose, branching out.	*Hormiscium* sp. 2
Root	7.5GY 5/6	Yes	No	Hypha septatebranched formed phyalid, conidia were globose, green	*Trichoderma* sp. 2
	N9	No	Yes	Hypha septate and nonseptate (unicell), hyaline	*Phytium* sp.
	N4	No	No	Hypha septate, brownish, branched at right and acute angles to the main hypha, not produce spore.	*Rhizoctonia* sp.
	2.5Y 5/4	No	No	Hypha septate	Unidentified 1 (no the isolated figure)

multinucleate *Rhizoctonia* was brown. An isolate of *Rhizoctonia* had a similar color with *Pythium* (white). Therefore, the determination of isolates required the micro morphological characteristics, such as spores and chlamydospores.

Endophytic fungi that were isolated from *P. falcataria*, showed a similar of the genus with fungi isolated by Saithong *et al.* (2010). They isolated and identified endophytic isolates from *Cephalotaxus mannii.* The endophytic fungi were *Cladosporium* sp., *Acremonium* sp., *Trichoderma* sp., *Monilia* sp., *Fusarium* sp., *Spicaria* sp., *Humicola* sp., *Rhizoctonia* sp., *Cephalosporium* sp., *Botrytis* sp., *Penicillium* sp., and *Geotrichum* sp. *Acremonium* sp. The other of research showed that *Trichoderma, Fusarium, Cladosporium* (Rosa *et al.* 2010; de Souza Sebastianes *et al.* 2013; Haddadderafhi *et al.* 2016) are the cosmopolitan fungi with a large abundance that can colonize some plants (non-specific host). This proved that *Aspergillus, Trichoderma,* and *Acremonium* from

Table 2 The relative abundance of endophytic fungi colonized leaf, twig, and root

Tissue plant segment	Species	The numberof isolates	The relative abundance (%)
Leaf	*Aspergillus* sp.	2	13.33
	Acremonium sp.	3	20.00
	Cladosporium sp.	4	26.67
	Trichoderma sp. 1	6	40.00
Twig	*Aspergillus* sp.	1	14.29
	Hormiscium sp. 1	3	42.86
	Acremonium sp.	1	14.29
	Hormiscium sp. 2	2	28.57
Root	*Pythium* sp.	2	22.22
	Rhizoctonia sp.	2	22.22
	Unidentified 1	2	22.22
	Trichoderma sp. 2	3	33.33

Table 3 The diversity index of endophytic fungi colonized leaves, twigs, and roots of sengon

	Leaves	Twigs	Roots
H' (Shanon-wiener index)	1.3095	1.2770	1.3050
E (Evenness index)	0.9446	0.9212	0.9414
C (Dominance index)	0.2758	0.3061	0.2593

Table 4 The IAA concentration from endophytic fungi

No.	Genus	Concentration IAA	Absorbance
1	*Cladosporium* sp.	311.3636364	0.31980
2	*Trichoderma* sp. 1	200.5090909	0.19786
3	*Trichoderma* sp. 2	64.21818182	0.04794
4	*Pythium* sp.	125.2818182	0.11511
5	*Rhizoctonia* sp.	51.97272727	0.03447
6	*Aspergillus* sp.	185.3181818	0.18115
7	*Acremonium* sp.	191.2909091	0.18772
8	Unidentified	123.9272727	0.11362
9	*Hormiscium* sp. 2	118.5454545	0.10770
10	*Hormiscium* sp. 1	72.01818182	0.05652

P. falcataria were the C-endophytes (clavicipitaceous) group. Whereas other isolates that have an uneven abundance in all parts of the plants isolated (Fig. 1 and Table 2), were Non-clavicipitaceous (NC-) endophytes group. Gazis and Chaverri (2010) explained that C-endophytes (clavicipitaceous) usually infest the host plant as one dominant fungal isolate/genotype and are vertically transmitted from maternal plants to offspring via seed infection. They confer benefits to the host in a host-specific manner which also depends on environmental conditions. Non-clavicipitaceous (NC-) endophytes display less host-specificity and are divided into three subclasses according to their biodiversity and transmission route (vertical or horizontal).

Evenness of fungi species in all of the *P. falcataria* parts that were isolated was high. The species diversity explained the dominance of fungi species. The Dominance index of fungi species was close to 1. This indicated that the dominance pattern was centralized, whereas if the dominance index is close to 0, it indicates that no species dominate in its community

(Odum 1993). According to it, the results showed that there was no concentration of dominance to one endophytic fungi species (Table 3).

The diversity of endophytic fungi from mangrove (Li *et al.*2016) and tea (Win *et al.* 2018) were higher in the twigs than in the leaves. Pawtowska *et al.* (2014) proved that the abundance of endophytic fungi in roots was lower than in the twigs but higher than in the leaves of *Lycopodium.* The results showed that diversity of endophytic fungi in leaves, twigs, and roots was at a moderate level. Thus, there were evenness species in all parts of *P. falcataria* that isolated, without any of the species dominating. The diversity of endophytic fungi is determined by some factors, such as a host species or cultivar, a host habitat, soil, geographic coordinates and tissues or organs of the host plants (colonization area) (Arnold and Lutzoni, 2007; Tian *et al.* 2004). Besides the environment factor, Haddadderafshi *et al.* (2016) explained that plant management has a major influence in determining abundance and diversity of endophytic fungi species. One of them is a canopy (Unterseher *et al.*2007; Scholtysik *et al.* 2013).

Production of IAA by Endophytic Fungi. Production of IAA from endophytic fungi was influenced by fungi species. Syamsiaa *et al.* (2015) described that the IAA production by endophyte varied, from 0.635 to 2.651 mg l^{-1}, depended on the fungi species. However, increased phytohormone production can be achieved by changing the growths condition in vitro, such as pH of growth media and nutrition. Waqas *et al.* (2014) said that the potential of endophyte in producing bioactive compound (phytohormone) is determined by nutrition, pH of the growth media, and genetically factor (Waqas *et al.* 2012). The relationship between IAA with pH media was when IAA is released under in vitro conditions, and becoming major cause of pH decrease, or IAA accumulation is directly proportional to pH decrease (Fu *et al.* 2015). But, apparently not all genera *Cladosporium* can produce IAA. Khan *et al.* (2017) found that IAA did not secrete by *Cladosporium* sp. but it was secreted by *Phoma* sp.

Secretion IAA has the related between abundance with fungi species that produced IAA. This is explained with the existence of genus *Cladosporium* that is found more as an endophyte in the plant (Paul and Yu, 2008; Bensaci *et al.* 2015; Sharma and Roy, 2015; Khan *et al.* 2017) than as a pathogen. When *Cladosporium* produce and secrete IAA so it can exert stimulatory and inhibitory effects on other fungi. It's mean that IAA is a major factor that determines the competition between fungal species that occupy the same niche (Fua *et al.* 2015).

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