DIVERSITY OF ENDOPHYTIC FUNGI IN THE ROOT, LEAF, STOLON AND PETIOLE OF ASIATIC PENNYWORT (*Centella asiatica*)

*Keragaman Cendawan Endofit pada Akar, Daun, Stolon dan Tangkai Daun Pegagan (*Centella asiatica*)*

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ABSTRAK/ABSTRACT

Endophytic fungi live in healthy tissues of many plants, including in medicinal plant such as Asiatic pennywort (*Centella asiatica*). These fungi exist in different parts of the plant as symbionts. The study aimed to isolate endophytic fungi from various parts of Asiatic pennywort of Malaysia accession and characterize their nature. Three individual plants of Asiatic pennywort (3 months-old) were obtained from the Sringanis Medicinal Garden in Bogor. The endophytes were isolated on Malt Extract Agar. The community structures of the endophytes were analyzed based on their diversity, colonization, dominance index, and relative frequency of occurrence of the isolated endophytic fungi. A total of 78 isolates have been obtained from three individual plants and clustered into 22 morphotypes consisted of 18 morphotypes of Ascomycota and 4 morphotypes of Basidiomycota divisions. The stolons harbored more endophytes (22.9 %) followed by leaf (16.7 %), root (11.8 %), and petiole (7.6 %). The diversity index was classified as medium category with the highest result (1.91) was found in the root, followed by leaf (1.79), stolon (1.75), and petiole (1.29). The most dominant endophytes were identified as *Ceratobasidium* sp., *Colletotrichum* sp, and *Fusarium* sp. *Ceratobasidium* sp. has the highest dominance index (0.02). UPGMA cluster analysis grouped the endophytic fungi into distinct clusters based on the plant parts origin. This study implied that stolon was the the most suitable part of Asiatic pennywort for isolating endophytic fungi. Further study is required to examine the role of the endophytic fungi to produce secondary metabolites in Asiatic pennywort.

*Cendawan endofit hidup di dalam jaringan tanaman yang sehat, termasuk tanaman obat seperti pegagan (*Centella asiatica*). Cendawan ini hidup di berbagai bagian tanaman sebagai simbion. Penelitian bertujuan untuk mengisolasi cendawan endofit dari berbagai organ (akar, daun, stolon dan tangkai daun) pegagan aksesi Malaysia dan mengkarakterisasi tingkat kolonisasi, indeks keanekaragaman, dominansi, dan frekuensi kehadiran relatif. Tiga individu tanaman pegagan berumur 3 bulan diperoleh dari Kebun Obat Sringanis Bogor. Cendawan endofit diisolasi pada media Malt Ekstrak Agar. Struktur komunitas endofit dianalisis melalui indeks keanekaragaman, kolonisasi, dominansi, dan frekuensi kehadiran cendawan endofit terisolasi. Sebanyak 78 isolat cendawan endofit telah diisolasi dari tiga individu tanaman dan dikelompokkan dalam 22 morfotipe, terdiri atas 18 morfotipe divisi Ascomycota dan 4 morfotipe divisi Basidiomycota. Bagian stolon diinfeksi lebih banyak oleh cendawan endofit (22,9 %) diikuti daun (16,7 %), akar (11,8 %), dan tangkai daun (7,6 %). Indeks keanekaragaman menunjukkan kategori sedang dengan nilai tertinggi (1,91) ditemukan pada akar, diikuti daun (1,79), stolon (1,75) dan tangkai daun (1,29). Cendawan endofit yang mendominasi diidentifikasi sebagai* Ceratobasidium *sp.,* Colletotrichum *sp., dan* Fusarium *sp. Indeks dominasi*

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menunjukkan Ceratobasidium *sp. memiliki nilai tertinggi (0,02). Analisis kluster dengan metode UPGMA mengelompokkan endofit ke dalam grup yang berbeda berdasarkan asal organ tanaman. Penelitian ini memberikan implikasi bahwa bagian stolon merupakan organ yang paling baik untuk isolasi cendawan endofit, pada pegagan. Studi lebih lanjut diperlukan untuk mengetahui peran cendawan endofit dalam menghasilkan metabolit sekunder pada pegagan.*

INTRODUCTION

The endophytic fungi are microorganisms that live in plant tissues without causing negative effects and even have symbiotic mutualism (Jia *et al*. 2016). The endophytic fungi play various roles in the host plants, such as enhancing plant growth as well as improving host plant resistance to biotic and abiotic stresses (Jia *et al*. 2016). In medicinal plants, several types of endophytic fungi are associated with bioactive compounds produced by the host plants (Devi *et al*. 2012; Jia *et al*. 2016).

Asiatic pennywort (*Centella asiatica*) is extensively used in traditional medicines. Many studies have been performed on the phytochemicals and the clinical properties of the plant (Devi *et al*. 2012; Joshi dan Chaturvedi 2013; Devi dan Prabakaran 2014). It contains bioactive compounds with therapeutic effects, such as wound healing activities, memory enhancement, neuroprotective, immune system regulator, anti-depressant, autoimmune prevention, anti-cancer, anti-diabetic, working enhancement of heart, blood vessels, and liver (Joshi dan Chaturvedi 2013).

As a medicinal plant, Asiatic pennywort is also known to be associated with various endophytic fungi. However, the studies on the endophytic fungi associated with Asiatic pennywort are still limited. For example, Malaysian accession has a high asiaticoside content (0.80 %) (Clay dan Holah 1999), however it differs from local accessions based on their morphological characters (Dahono 2014). These differences may accommodate different microbial diversity, including endophytic fungi. Nalini *et al*. (2014) stated several isolated endophytic fungi were associated with the roots, flower stalks, and stolon of the Indian Asiatic pennywort. Rakotoniriana (2012) have also isolated several endophytic fungi from the leaf of Madagascar Asiatic pennywort and *Colletotrichum* sp. was identified as the most dominant species.

Current studies revealed that microorganisms, including endophytic fungi, may contribute to the production of secondary metabolites in medicinal plants (Stierle *et al*. 1993; Venugopalan dan Srivastava 2015). Taxol and taxane were the first metabolites produced by *Taxomyces andreanae*, an endophytic fungus of Pacific yew (*Taxus brevifolia*) (Stierle *et al*. 1993. Since then, studies on the potential roles of endophytic fungi *in vitro* production of plant secondary metabolites have become more feasible (Venugopalan dan Srivastava 2015).

The study on the fungal diversity and distribution associated with Asiatic pennywort of Malaysian accession is limited. Therefore, the present study aimed to isolate endophytic fungi from various parts (root, leaf, stolon, and petiole) of Asiatic pennywort of Malaysian accession and characterize their nature. The study was expected to support the development of effective methods to produce asiaticoside from the non-host plant.

MATERIALS AND METHODS

Plant materials

The 3 months old of three individual healthy plants of Asiatic pennywort of Malaysian accession were obtained from the Sringanis Medicinal Garden in Bogor, West Java ($6^{o}38'13.7"S$ $106^{o}48'57.2"E$). The experiments were conducted at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) and the Center for Integrated Laboratory of Syarif Hidayatullah Islamic University Jakarta.

Isolation and identification of endophytic fungi

All plants were uprooted and cleaned from debris. A total of 36 samples of plant parts (3 replications from each organ) were taken from the three individual plants. Each plant part was then cut into 4 segments with 1 cm x 0.5 cm in size, hence there were 144 segments.

The samples were then immediately processed for endophytic fungi isolation. The endophytic fungi were isolated following (Hidayat *et al*. 2016) method. The leaves, petioles, stolons, and roots were washed in the running tap water for 10 minutes, surface-sterilized using 70 % ethanol for 1 minute. The samples were then soaked in 3 % sodium hypochlorite for 2 minutes and in 70 % ethanol for 20 seconds, rinsed three times in sterile distilled water and dried on the sterile paper for at least 4 hours. The final rinse of the samples (100 µl) was poured onto the agar medium as a quality control of sterilization process, if there were no fungi grown on the medium, meant the surfaced-sterilization process was successful.

Each sterile plant sample was cut approximately into 1 cm × 0.5 cm in size and cultured in Malt Extract Agar (MEA) (Difco, USA). The cultures were incubated at a room temperature for 30 days. The endophytic fungi grown on the MEA medium were observed every day until 30 days after the incubation. The endophytic fungi grew on the 1st day until the 14th days were classified into the fast-growing fungi, whereas the ones that grew after 14 days were classified into the slow-growing fungi (Bosshard 2011). The fungal cultures were kept in the Biogen Culture Collection, ICABIOGRAD.

The morphological characters of the endophytic fungi isolates were classified based on their color, shape, and diameter of growth. The colonies with similar characteristics were grouped into the same morphotype (Putra *et al*. 2015; Radiastuti 2015).

Following the initial morphological characterization, the endophytic fungal isolates were examined macroscopically and micros-copically. The macroscopic observations were the morphological shape, color of the top and bottom side of the colonies, colony diameter, colony elevation, colony surface texture, mycelium type, colony edge, colony density, colony zoning, the presence of exudates, and the presence of concentric radial lines on the surface of the colony. The microscopic observations, using a light microscope at 400x and 1000x magnification, were the hyphae (septation), shape and size of spores/conidia, conidiophore, conidio cells, and the presence of rhizoid. The morphotypes of endophytic fungi were identified following the standard identification books (Barnett dan Hunter 1998; Crous *et al*. 2009; Radiastuti 2015).

Data analysis

The community structures of the endophytic fungi were analyzed based on diversity, colonization, dominance index, and frequency of occurrence of endophytic fungi isolated from each plant parts. One colony represented an individual endophytic cell.

Colonization

The colonization was calculated based on (Petrini dan Fisher 1988) formula as follows :

$$\text{Colonization (\%)} = \frac{\text{Total number of segments colonized by endophytic fungi}}{\text{Total number of segments observed}} \times 100\%$$

Frequency Relative of Occurrence

The frequency relative of occurrence of endophytic fungi species was calculated to obtain the distribution value of endophytic fungi species from various organs, using the formula as follows (Radiastuti 2015):

$$\text{Frequency relative of occurrence } i \text{ (FR)} = \frac{\text{Numbers of strain in species} - i}{\text{Total number of strain found}} \times 100\%$$

Diversity Index

The Shannon-Wiener diversity index (H') presents the levels of diversity (high, medium, and low) and compares the diversity of endophytic fungi amongst various organ of Asiatic pennywort. The H index is calculated based on the formula as follows (Tao *et al*. 2012):

$$H' = -\sum_{i=1}^{k} pi \times \ln pi$$

H' = Shannon-Wiener diversity index/ *Indeks keanekaragaman Shannon-Wiener.*

Pi = $\frac{ni}{N}$ = Proportion of total number of individual for each species/*Proporsi jumlah total individu untuk setiap spesies.*

Ni = Number of total individual for each species/*Jumlah total individu untuk setiap spesies.*

N = Number of all individuals/*Jumlah semua individu.*

The Criteria of Shannon-Wiener diversity index/ *Kriteria indeks keanekaragaman Shannon-Wiener*:

H' < 1 : low level of diversity/*tingkat keaneka-ragaman yang rendah*,.

1 < H' < 3 medium level of diversity/*tingkat keanekaragaman sedang.*

H' > 3 : high level of diversity/*tingkat keaneka-ragaman yang tinggi.*

Dominance index

The Simpson Dominance Index was used to analyze the presence of endophytic fungi species that dominate the community of Asiatic pennywort of Malaysian accession. The formula used to assess the Dominance Index was as follows (Odum 1996):

$$C = \sum (Pi)^2$$

C = Shannon-Wiener Diversity Index/*Indeks Keragaman Shannon-Wiener*.

Pi = $\frac{ni}{N}$ = Proportion of total number of individual for each species/*Proporsi jumlah total individu untuk setiap spesies*.

ni = Number of total individual for each species/*Jumlah total individu untuk setiap spesies.*

N = Number of all individuals/*Jumlah semua individu*.

The Criteria of Dominance Index/*Kriteria Indeks Dominasi* :

0,01 < C < 0,30 : low level of dominance/*dominasi tingkat rendah*.

0,31 < C < 0,60 : medium level of dominance/*dominasi tingkat menengah*.

0,61 < C < 1,00 : high level of dominance/*dominasi tingkat tinggi.*

UPGMA Analysis

The cluster analysis and the relative frequency of the endophytic fungi presence was performed using the UPGMA method (Unweighted Pair Group Method Using Arithmetic Mean). The similarity index was determined using Jaccard's Coefficient on MVSP computer program version 3.22 (Hilarino *et al*. 2011). Index values ranged between 0-1, if the value close to 1 indicated the higher level of species similarity (Ludwig dan Reynold 1988). The dendogram represented the relationship between the endophytic fungi community structure and the plant organs, determined by the similarity index in the distance matrix.

RESULTS AND DISCUSSION

Endophytic fungi distribution

Eighty five fungal endophytes were isolated from the samples of Asiatic pennywort of Malaysian accession. The fungi consisted of 24 isolates from the leaves, 17 isolates from the roots, 11 isolates from the petioles, and 33 isolates from the stolons (Table 1). The endophytic fungi were further grouped into 23 morphotypes (Table 2). The distribution of endophytic fungi from different parts of the plant was varied. The number of endophytic fungi colonized the stolon were higher than the roots, leaves, and petioles.

The distribution of endophytic fungi in the host plants can be associated with several factors, such as the origin of the colonized endophytes and the presence of particular substances in the plant organ tissues. It might be related to the ability of each endophytic fungal species to utilize particular substrates or plant tissues. Jia *et al*. (2016) stated that endophytic fungi colonization was significantly determined by plant tissues that produce a variety of substances. Furthermore, the different endophytic fungi composition in different host organs can occur due to its histologic differences and nutrients availability in the plant organ in which endophytic fungi colonized (Arnold dan Lutzoni 2007). Further, Arnold *et al*. (2001) suggested that different leaves in the same tree might have distinct endophytic colonies.

Colonization rate

Amongst 144 segments of Asiatic pennywort, the colonization rate of endophytic fungi in the stolons, leaves, petioles, and roots was 59 %. This implied that almost half of the plant segments (59 %) were colonized by endophytic fungi. The stolon segments harbored more fungal endophytes (22.9 %), followed by the leaves (16.7 %), roots (11.8 %), and petioles (7.6 %) (Table 1). The richness of the stolon segments colonized by the endophytic fungi might be associated with its higher biomass content that allowed more niches to be colonized than other organs. Most of the isolated endophytic fungi were categorized as fast- and slow-growing fungi.

Table 1. Colonization rate of endophytic fungi in various segments of Asiatic pennywort of Malaysian accession.
Tabel 1. Segmen terkolonisasi dan tingkat kolonisasi cendawan endofit pada berbagai organ pegagan aksesi Malaysia.

Plant parts	Number of samples	Number of colonized samples	Colonization rate (%)
Leaf	36	24	16.7
Root	36	17	11.8
Petiole	36	11	7.6
Stolon	36	33	22.9

Previously, Rakotoniriana *et al*. (2008) found that 78 % of leaves of Asiatic pennywort of Madagascar were colonized by endophytic fungi. Colonization percentage of endophytic fungi in Asiatic pennywort was apparently associated with climatic conditions as reported by (Gupta dan Chaturvedi 2017). They revealed that in the rainy season, more endophytic fungi was isolated (38.37 %) than in the summer (26.37 %) and winter (15.40 %). However, Gong dan Guo (2009) showed that a higher colonization rate of fungi was found in the stems than in the leaves of *Dracaena cambodiana* and *Aquilaria sinensis*. The distribution of endophytic fungi in various plant organs can be influenced by several factors such as by the plant environment. Wu *et al*. (2013) mentioned that environmental conditions was an important factor in determining the type and number of secondary metabolites of the host plants and also affected the structure of endophytic fungal

Table 2. Morphotypes of endophytic fungi isolated from different parts of Asiatic pennywort of Malaysian accession.
Tabel 2. Jumlah dan jenis isolat cendawan endofit yang ditemukan pada berbagai organ pegagan aksesi Malaysia.

Morphotype -	Spesies	Class	Segment of Asiatic pennywort				Plant – (1/2/3)*
			Leaf (L)	Root (R)	Petiole (P)	Stolon (S)	
1. MM 8	*Phialemoniopsis* sp.	Sordariomycetes	-	4	-	-	R(3)
2. MM 13	*Aspergillus* sp.	Eurotiomycetes	-	2	-	-	R(3)
3. MM 1	*Ceratobasidium* sp.	Agaricomycetes	-	3	-	9	R (3), S (1,2)
4. MM 19	*Chaetomium globosum*	Sordariomycetes	-	1	-	-	R (2)
5. MM 18	*Colletotrichum tabaci* 1	Sordariomycetes	1	-	-		L (2)
6. MM 23	*Colletotrichum tabaci* 2	Sordariomycetes	-	4	3	-	R (3), P (1)
7. MM 14	*Colletotrichum gigasporium*	Sordariomycetes	1	-	-	-	L (2)
8. MM 9	*Colletotrichum siamense*	Sordariomycetes	3	-	-	-	L (2)
9. MM 2	*Colletotrichum karstii*	Sordariomycetes	10	-	-	-	L (1)
10. MM 3	*Fusarium solani* 1	Sordariomycetes	1	-	-	6	L (2), S (1)
11. MM 4	*Fusarium* sp.1	Sordariomycetes	-	-	-	6	S (1,2)
12. MM 17	*Fusarium solani* 2	Sordariomycetes	-	-	-	7	S (1)
13. MM 22	*Fusarium* sp.2	Sordariomycetes	-	-	-	2	S (1,3)
14. MM 20	*Fusarium striatum*	Sordariomycetes	-	-	-	1	S (3)
15. MM 5	*Eutypella* sp.	Sordariomycetes	-	1	-	-	R (3)
16. MM 6	*Trametes* sp.	Agaricomycetes	-	-	3	-	P (2)
17. MM 10	*Peroneutypa scoparia*	Sordariomycetes	-	1	-	-	R (3)
18. MM 15	*Penicillium capsulatum*	Eurotiomycetes	2	-	-	-	L (2,3)
19. MM 21	*Perenniporia corticola*	Agaricomycetes	4	-	-	-	L (2)
20. MM 12	*Phanerochaete* sp.	Agaricomycetes	1	1	-	-	L (1), R (3)
21. MM 11	*Phomopsis asparagi*	Sordariomycetes	1	-	1	-	L(2), P(2)
22. MM 7	*Phyllosticta* sp.	Dothideomycetes	-	-	4		P(2)
23. MM 16	*Talaromyces* sp.	Eurotiomycetes	-	-	-	2	S(2)
Total			**24**	**17**	**11**	**33**	

Note/*Keterangan* : *) 1,2,3 = 1st, 2nd, and 3rd individual plants/*individu tanaman.*

population of the host plant. The ability of endophytic fungi to colonize plant parts of Asiatic pennywort indicated that plant was suitable to support endophytic fungi infestation. Some endophytic fungi also promoted secondary metabolites of the host plants (Shwab dan Keller 2008).

Diversity index

The results showed that the H' index amongst the organs was not different. The H' value of root was 1.91, leaf (H'=1.79), stolon (H'=1.75) and petiole (H'=1.29) and were classified as medium diversity (Figure 1). The diversity index of root and leaf were higher than the stolon and petiole. This indicated that the number of endophytic fungi species obtained from the root (8 species) and the leaf (9 species) was higher than from the stolon (7 species) and petiole (4 species).

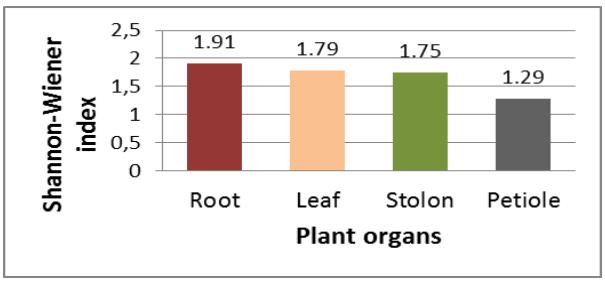

Figure 1. The Shannon-Wiener Diversity Index (H ') of endophytic fungi in the various organs of Asiatic pennywort of Malaysian accession.

Gambar 1. Indeks keanekaragaman Shannon-Wiener (H') cendawan endofit pada berbagai organ pegagan aksesi Malaysia.

The root has the highest index diversity compared to the other parts of Asiatic pennywort of Malaysian accession which lead to a high diversity of endophytic fungi. Root has the most widespread surface, hence its possesses more possibility to be in contact with the environment. The plant could produce root exudates, which play an important role in modifying the complexity and dynamic of the environment (Xiao *et al*. 2014). The roots were also inhabited by various microorganisms and became a medium for spores and microorganisms to spread across the plant organs (Arnold 2007). The fungal endophytes obtained from the root were more diverse than leaves with H'value at 1.71 (Haddadderafshi 2015).

The leaves showed the second highest H' index value (1.79), and have the same level of diversity index (medium level) as reported in other study (H'=1.97) (Gupta dan Chaturvedi 2017). Similar results were also reported on the endophytic fungi isolated from *Piper nigrum* in which the root had higher H' value (H'=1.33) than leaf (H'=0.69) and petiole (H'=0.69) (Uzma *et al*. 2016).

Dominance index

The dominance index (D) of *Ceratobasidium* sp. endophyte was the highest (D = 0.020) (Table 3). A total 12 out of 78 fungal endophytes was identified as *Ceratobasidium* sp. isolated from stolon and root, followed by *Colletorichum karstii* (D = 0.014), *C. tabacci* (D = 0.007), and *F. solani* 1 (D = 0.007) (Table 3). The *Ceratobasidium* sp. was a common endophytic fungi and known to be associated with roots in

Table 3. Dominance index (D) of endophytic fungi in the various organs of Asiatic pennywort of Malaysian accession.

Tabel 3. Indeks Dominansi (D) cendawan endofit pada berbagai organ pegagan aksesi Malaysia.

Endophytic fungi	Dominance Index
Ceratobasidium sp.	0.020
Colletotrichum karstii	0.014
Colletotrichum tabaci 2	0.007
Fusarium solani 1	0.007
Fusarium solani 2	0.007
Fusarium sp. 1	0.005
Phialemoniopsis sp.	0.002
Phyllosticta sp.	0.002
Perenniporia corticola	0.002
Colletotrichum siamense	0.001
Trametes sp.	0.001
Aspergillus sp.	0.001
Fusarium sp. 2	0.001
Penicillium capsulatum	0.001
Phanerochaete sp.	0.001
Phomopsis asparagi	0.001
Talaromyces sp.	0.001
Chaetomium globosum	0.000
Colletotrichum tabaci 1	0.000
Colletotrichum gigasporum	0.000
Eutypella sp.	0.000
Peroneutypa scoparia	0.000
Fusarium striatum	0.000

various plants (Matsumoto 2005; Irwin *et al*. 2007). However, *Colletotrichum* and *Fusarium* had better adaptation to the environmental conditions. Therefore, it is easy to find these fungi in various host plants. Other studies reported that *Colletotrichum* and *Fusarium* were the most common endophyte found in various medicinal plants, such as *Polygonum acuminatum* and *Aeschynomene fluminensis* (Souza *et al*. 2017), as well as *Echinacea purpurea* (Rosa *et al*. 2012). Other species of endopytic fungi found in Asiatic pennywort of Malaysian accession were *Phillosticta capitalensis, Acremonium* sp., *Phomopsis asparagi, Aspergillus flavus, Penicillium capsulatum, Talaromyces* sp. and *Chaetomium globosum* (Syed *et al*. 2009; Vinale *et al*. 2017; Wikee *et al*. 2013; Nair dan Padmavathy 2014; Russo *et al.* 2016; Supriya dan Audipudi 2015).

Relative frequency

The highest level of relative frequency of endophytic fungi was presence in the stolon (38.83 %), followed by leaf (28.25 %), root (20.02 %), and petiole (12.77 %) (Figure 2). The stolon were colonized by 33 isolates (consisted of 3 genera and 5 species); followed by the leaf with 24 isolates (6 genera and 9 species), root with 17 isolates (8 genera and 8 species), and the lowest one was the petiole with 11 isolates (over 4 genera and 4 species).

The stolon organs were dominated by *Ceratobasidium* sp. (10.59 %) which was also present in the root (3.53 %) (Figure 2). Endophytic fungi obtained from the stolon, such as *Ceratobasidium* sp., *Fusarium solani, Talaromyces* sp., and *Fusarium* sp. have never been reported. *Ceratobasidium* sp. is a telomorphic form of *Rhizoctonia* sp., endophytic fungal known to be associated with the plant roots. Some of the *Ceratobasidium* species, such as *C. cornigeum, C. setariae, C. gramineum,* and *C. oryzae-sativa* were known to be associated with *Rhizoctonia* sp. in the plant roots (Matsumoto 2005). *Ceratobasidium* was also reported to be associated with the root of *Pterostylis nutans* (Irwin *et al*. 2007).

Colletotrichum kartsii in the leaf has the highest relative frequency (11.76 %), followed by *Perenniporia corticola* (4.71 %), and *C. siamense* (3.53 %) (Figure 2). *Colletotrichum* sp., *C. kartsii, Fusarium solani, Penicillium capsulatum, Phomopsis asparagi, Phaerochaete* sp. and *Perenniporia* sp., retrieved from the leaves have not been reported to be existed in the leaf of Asiatic pennywort of Malaysian accession. A similar study revealed the dominance of *Colletotrichum* in the stem and leaf of medicinal plants. However, the domination of *Xylariaceae* sp. and *Colletotrichum higginsianum* was found in the leaves of Asiatic pennywort from Madagascar (Rakotoniriana 2012). Similar study also revealed the highest level of relative frequency of occurrence from the leaves and the stems of the medicinal plants in China (31.3 %) and from the leaves of *Taxus x media* (58.6 %) (Huang *et al*. 2008; Xiong *et al*. 2013).

The *Colletotrichum* sp. are found in all plant organs, meaning that they were non organ-specific fungi. They commonly found as symbiont in host plants, *i.e*. mutualism, antagonists and pathogens. The *Colletotrichum* is recognized by its ability to alter their mechanisms of life style, not only as endophytes, but also possessed necrotropic mechanisms (damaging host tissue), biotropics (getting nutrients without damaging the host) or passively live in the host plants. These changes occur due to alteration of conditions in plant physiology, environment, and plants genotypes (Silvia *et al*. 2017).

The highest level of relative frequency of occurrence in the root was indicated by *Phialemoniopsis* sp. and *Colletotrichum tabaci* 2, at 4.71 %, followed by *Ceratobasidium* sp. (3.53 %) (Figure 2). In previous study, some unreported endophytic fungi such as *Colletotrichum* sp., *P. capsulatum, Eutypella* sp., and *Ceratobasidium* sp. were isolated from the roots of Asiatic pennywort of Malaysian accession. Nalini *et al.* (2014) isolated *Acremonium* sp. from the root and stolon of Asiatic pennywort from

India. *Acremonium* and *Ceratobasidium* are known as root-endophyte (Matsumoto 2005; Irwin *et al*. 2007; Stocker dan Alten 2016). Specific endophytic fungi *A. oryzae* was also obtained from the root. In the previous study, *Aspergillus* was also found in the root of Asiatic pennywort (Nath *et al*. 2014) and maize (Russo *et al.* 2016).

Four species of endophytic fungi were obtained from the petiole in which *Phylosticta capitalensis* indicated as the highest percentage of relative frequency of occurence (4.71 %) (Figure 2). *P. capitalensis* is a common endophyte which colonized a number of plants (Wikee *et al*. 2013). The other fungi species which colonized the petiole were *Tremetes* sp. (3.53 %), *Colletotrichum tabaci* 2 (3.35 %) and *Phomopsis asparagi* (1.18 %). All the fungi species mentioned above have never been reported to be colonized in the petioles of Asiatic pennywort, especially of Malaysian accession.

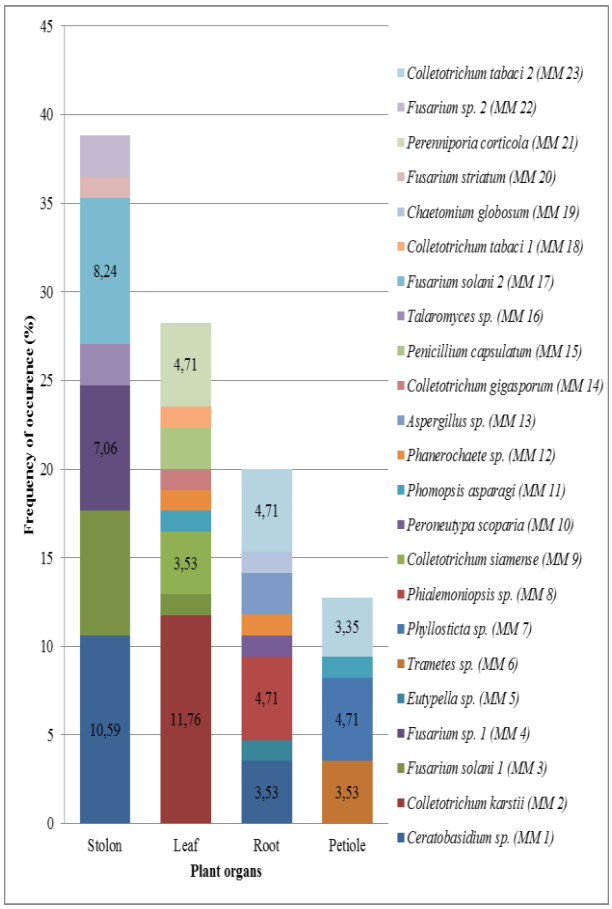

Figure 2. Relative frequency of occurence of endophytic fungi in various plant organs of Asiatic pennywort of Malaysian accession.

Gambar 2. Frekuensi kehadiran relatif cendawan endofit pada berbagai organ pegagan aksesi Malaysia

Almost of the endophytic fungi were found as organ-specific, except *Ceratobasidium* sp. which was found in the stolon and root. The relative frequency of occurence of *Ceratobasidium* sp was higher in stolon (10.59 %) than root (3.53 %) (Figure 2).

Cluster Analysis of endophytic fungi communities in Asiatic pennywort organs of Malaysian accession

The similarity index describes the level of similarity in the structure and species composition of endophytic fungi in various Asiatic pennywort plant organs of Malaysian accession. Based on the UPGMA analysis, the endophytic fungi of Asiatic pennywort of Malaysia accession was divided into three clusters with similarity index value <9.1 % (0.091) as a whole plant organs (Figure 3).

The similarity index value <10 % (0.100) indicated that the endophytic fungi community amongst various parts of Asiatic pennywort of Malaysian accession was different. For instance, *Colletotrichum gigasporum*, *C. karstii*, *C. siamense*, *C. tabaci* , *Penicillium capsulatum*, and *Perenniporia corticola* were only found in the leaf (Figure 2).

Each endophytic fungus species occupies a suitable habitation for its existence. The plant organs provide microhabitat suitable for the life of endophytic fungi. Microhabitat development was affected by chemical components (Xiao *et al*. 2014). The endophytic fungi community in the petiole was closer to the root than to other organs, and was grouped as the first cluster with similarity index (IS) 10% (0.100). The community found on the stolon has a level of similarity with the leaf (the second cluster), with IS at 6.7% (0.067). Based on the endophytic fungi community, petioles and root (node 1) and stolon and leaf (node 2) had IS of 3.3% (0.033) (Figure 3).

The present study suggested that Asiatic pennywort was rich in endophytic fungi and the stolon harbored the most richness endophytic fungi

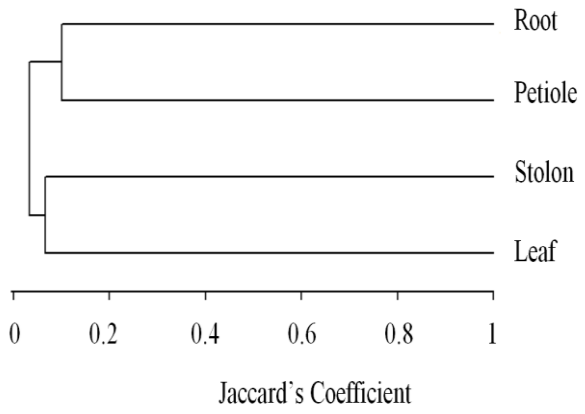

Figure 3. Dendogram of endophytic fungi community in various organs of Asiatic pennywort of Malaysian accession

Gambar 3. Dendogram komunitas cendawan endofit pada berbagai organ pegagan aksesi Malaysia

community. Four endophytic fungi identified from the stolon were *Fusarium solani* 2, *F. solani* 3, *F. striatum* and *Talaromyces* sp. Further study are required to investigate the secondary metabolites produced by the fungi as well as the role of the endophytic fungi to improve secondary metabolites in Asiatic pennywort.

CONCLUSION

Various endophytic fungi were colonized the different parts of Asiatic pennywort. The diversity of the endophytic fungi was classified as a medium diversity. The highest diversity of the endophytic fungi was obtained from the roots, followed by the leaf, stolon, and petiole. *Ceratobasidium* sp., *Colletotrichum* sp., *C. destructivum*, and *Fusarium solani* were the most dominant endophytic fungi in plant organs of Asiatic pennywort of Malaysian accession.

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