

REGULAR ARTICLE

ENDOPHYTIC FUNGI COMMUNITIES FROM *Centella asiatica* OF BENGKULU ACCESSIONNani Radiastuti^{*1}, Jeanne Isbeanny Laraswati¹, Dwi Ningsih Susilowati² and Nurhasni¹

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ABSTRACT

Various species of endophytic fungi have been isolated and reported from various types of plants and tissues, but how host or plant tissue determines endophytic community structure has not been explored quantitatively. The aim of this research was to understand community structure of endophytic fungi in various organs of *Centella asiatica* of Bengkulu accession. The analysis data use to Shannon Wiener diversity index (H'), dominance index, colonization rate, frequency of occurrence and UPGMA analysis with Jaccard Coefficient. Total 61 isolates were obtained and grouped into 19 morfotypes, consist of 15 *Ascomycetes* & 4 *Basidiomycetes* and 10 Genera. The highest colonization rate was in stolons (16%) and the lowest is in petioles (4%). Endophytic fungi were distributed in all of the organs. Leaves harbored more diverse endophytic fungi ($H' = 2,138$) than the other organs and classified as medium. *Fusarium solani*, *Fusarium oxysporum* dan *Phoma multirostrata* were the dominant species. This research showed that different type of host-plant organs influenced community structure of endophytic fungi from *C. asiatica* of Bengkulu accession. This is the first report, on the community structure of endophytic fungi from *C. asiatica* from leaves, petioles, stolons, and roots part of the organ plant. Overall, our results indicated that the isolate can be used to analyzed of it's potential as bioactive compound same as the host.

Keywords: Bengkulu accession, *Centella asiatica*, community structure, endophytic fungi

INTRODUCTION

Pegagan (*Centella asiatica* (L.) Urb.) is a medicinal plant that is widely used as a raw material for traditional medicinal herbs. The *C. asiatica* is widespread throughout tropical and subtropical countries world wide included in Indonesia (Gupta, 2013). The *C. asiatica* of Bengkulu accession is one type of pegagan plant that is cultivated in Indonesia widely and has superior agronomic character. The species is known to have many benefits for medicinal, such as a wound healing drug (Shetty *et al.*, 2008), anti-inflammatory (George *et al.*, 2010), antidiabetic, antitumor (Rao and Mastan, 2007), antibacterial (Zaidan *et al.*, 2006), antioxidants (Hamid *et al.*, 2002), cosmetic and human consumption (Gupta, 2013).

The *Centella asiatica* as a medicinal plant is known to have a symbiosis with endophytic fungi. Endophytic fungi are microorganisms that live in plant tissues by forming colonies and not parasitic in the host plants (Strobel and Daisy, 2003). The endophytic fungi have benefit if can live inside in the host plant e.i producing antibiotics (Sun *et al.*, 2008), enzymes (Bezerra *et al.*, 2015), antimicrobial substances and plant growth hormones (Hwang *et al.*, 2011). The endophytic fungi in *C. asiatica* plants are reported to contain cytotoxic and antitumor compounds (Devi and Prabakaran, 2014). The asiaticoside and madecassoside could be produced in the leaf and root of *C. asiatica* (Aziz *et al.*, 2007). Majority studies of endophytic fungi in the *C. asiatica* have focused more on isolation and screening of secondary metabolites.

Research on the community structure of endophytic fungi in medicinal plants in Indonesia has not been widely reported especially in the *C. asiatica*. While it is important to know the relationship between endophytic fungi and host plants. It is closely related to the production of secondary metabolites produced, knowing the diversity and function of endophytic fungi in the ecosystem (Hoffman and Arnold, 2008). Ginting *et al.* (2013) succeeded in isolating *Aspergillus sydowii*, *Colletotrichum higginsianum*, *Fusarium solani* and *Leptosphaerulina australis* from leaf organ of local *C. asiatica*, as well as *Glomerella cingulata*, *Mycotetradiscus indicus* and *Stagnosporopsis cucurbitacearum* from the *C. asiatica* of Malaysian accession. The community structure of the endophytic fungi of *C. asiatica* plants was studied by Rakotoniriana (2007) on leaf organs of *C. asiatica* plants from Madagascar and *Colletotrichum* sp. as the most dominant species. Meanwhile, the community structure in the several organ of *C. asiatica* of Bengkulu accession has never been reported. Information about the community structure of endophytic fungi in each organ can be a reference in more effective use to the diversity of endophytic fungi in the *C. asiatica*. Hence, it is essential to understand such as relationships between endophytic fungi and in their host plants. This objective of our study was determined the community structure of endophytic fungi in several organs of *C. asiatica* especially in the Bengkulu accession.

MATERIAL AND METHODS

Isolation

Isolation of fungal endophyte from four part of the *C. asiatica* namely leaves, petioles, stolons, and roots were collected from the plant collection BB Biogen (Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development). Three pieces of each healthy organ were taken from three individual plants. The fungal endophytes were isolated using isolation protocol described by Hidayat *et al.* (2016)

Identification of fungi

The isolates were grouped into morphotypes, based on their colony characteristics. Representative cultures from each morphotype were then selected for molecular analysis (data not shown in this study)

Data analysis

The community structure is expressed through diversity, colonization rate, dominance index and frequency of occurrence of endophytic fungi species in each plant organ. One colony is defined as an individual endophytic fungi

Colonization rate

The colonization formula is defined as follows:

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

Relative frequency of occurrence

The presence of endophytic fungi species in various plant organs is calculated using the formula as follows:

$$\text{Relative frequency of presence } i \text{ (FR)} = \frac{\text{Numbers of strain in species}-i}{\text{Total number of strain found}} \times 100\%$$

Diversity index

The H index is calculated based on the formula as follows (Tao *et al.*, 2012):

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

H' = Shannon-Wiener index

$P_i = \frac{n_i}{N}$ = Proportion of total number of individual for each species

ni = Number of total individual for each species
N = Number of all individuals

The Criteria of Shannon-Wiener Diversity Index is as follows:

$H' < 1$: low level of diversity
 $1 < H' < 3$: median level of diversity
 $H' > 3$: high level of diversity

Dominance index

The formula of Simpson Dominance Index is used to the Dominance Index (Odum, 1996):

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

C = Shannon-Wiener Diversity Index
 $P_i = \frac{ni}{N}$ = Proportion of total number of individual for each species
ni = Number of total individual for each species
N = Number of all individuals

Cluster analysis

The cluster analysis by dendrogram was conducting using the UPGMA method (Unweighted Pair Group Method Using Arithmetic Mean). The similarity index is analyzed using Jaccard's Coefficient. The dendrogram construction described the relationship between the endophytic fungi community structure and the plant organs, based on the similarity index in the distance matrix. The relative frequency of occurrence data was analyzed with the UPGMA method, using Jaccard's Coefficient on MVSP computer program version 3.22 (Hilarino et al., 2011)

RESULTS AND DISCUSSION

Number of endophytic fungi from *Centella asiatica*

The number of endophytic fungi that were isolated in the different organ of *C. asiatica* are successful. A total of 61 endophytic fungi from 144 segments of *C. asiatica* of Bengkulu accession. The isolates were obtained as much as 23, 20, 12, and 6 from stolons, roots, leaves and petiols respectively. As much as 61 fungal endophytes that colonized fragments and grouped into 18 morphotypes based on their similar morphological characteristics on Potato Dextrosa Agar (PDA). A total of 19 morphotypes were identified based on the ITS sequences data.

Table 1 Identified endophyte fungi from *Centella asiatica* of Bengkulu accession based on ITS r DNA

Endophytic fungi		Organ				
Divisi	Class	Leaf	Root	Petiol	Stolon	
Ascomycetes	<i>Dothidiomycetes</i>					
	<i>Acrocalymma aquatica</i>	1	2	-	-	
	<i>Phoma multirostrata</i>	1	3	-	3	
	<i>Eurotiomycetes</i>					
	<i>Aspergillus versicolor</i>	1	-	2	-	
	<i>Sordariomycetes</i>					
	<i>Colletotrichum</i> sp.1	2	-	-	-	
	<i>Colletotrichum</i> sp.2	2	-	2	2	
	<i>Fusarium oxysporum</i> 1	-	1	-	-	
	<i>F. oxysporum</i> 2	-	2	-	6	
	<i>F. oxysporum</i> 3	-	1	-	-	
	<i>F. falciforme</i> 1	-	2	-	2	
	<i>F. falciforme</i> 2	1	6	-	3	
	<i>F. falciforme</i> 3	-	1	-	1	
	<i>F. keratoplasticum</i>	-	1	-	2	
	<i>Mycochetophthora gentinae</i> 1	2	-	-	-	
	<i>Mycochetophthora gentinae</i> 2	1	-	-	-	
	Basidiomycetes	<i>Agariomycetes</i>				
		<i>Ceratiobasidium</i> sp.	-	1	-	3
<i>Phanerochaete chrysosporium</i>		-	-	2	-	
<i>Perenniporia</i> sp.		1	-	-	-	
<i>Trichaptum</i> sp.		-	-	-	1	
Total		12	20	6	23	

Legend: - no isolates

These endophytic fungi include *Aspergillus* sp., *Fusarium falciforme*, *F. oxysporum*, *F. keratoplasticum*, *Colletotrichum* sp., *Phoma multirostrata*, *Ceratobasidium* sp., *Acrocalymma vagum*, *Trichaptum* sp., *Perenniporia* sp., *Phanerochaete chrysosporium*, and *Mycochaetophora gentinae* (Table 1). The endophytic fungi were obtained from the Ascomycetes and Basidiomycetes division respectively, class Sordariomycetes (61.1%), Agaricomycetes (22.2%), Dothidiomycetes (11.1%), and Eurotiomycetes (5.5%). The results of these studies indicate the colonization rate reached 78%. The host organ provides a microhabitat that is suitable for the life of endophytic fungi that are symbiotic in the host plant. The plant is a suitable host for colonizing the endophytic fungi in the plant organs tissue. Therefore, the calculation of the level of endophytic fungi colonization in the host plant is carried out.

Colonization rate

The study is found only 42% (61 segments of organs) were colonized with endophytic fungi from a total of 144 segments of the organs examined. The segments is not colonized by endophytic fungi is 58% (Figure 1). The percentage of the segments aren't colonized higher than the segments that is colonized.

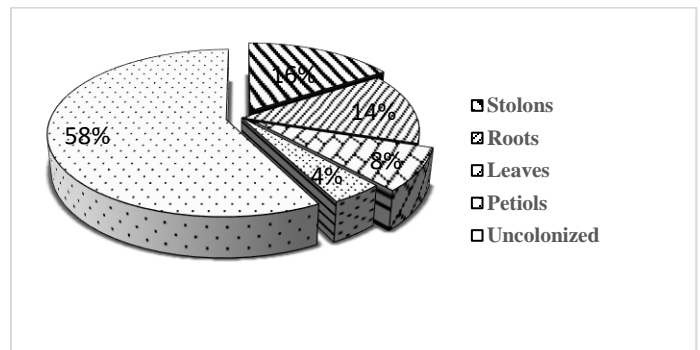


Figure 1 Colonization rate of endophytic fungi from *Centella asiatica* of Bengkulu accession

The level of colonization in each organ that was being observed (leaves, petiol, stolons and roots), showed a difference in the number of isolates that successfully isolated. The colonization rate of endophytic fungi was found higher in stolon (16%) than in root (14%), while in other organs such as leaves and petiols, 8% and 4% respectively (Figure 1). In each part of the organ, sometimes more than one endophytic fungi is found which colonizes the same organ segment. In general, this indicates that the level of colonization that occurs is higher in the lower plant organs, such as stolon and roots, compared to the upper plant organs (petiols and leaves).

The difference in the level of colonization of *C. asiatica* plants in accession of Bengkulu shows the success rate of endophytic fungi in colonizing plant organs. This study only the culturable isolate could be isolated. Based on the results, more endophytic fungi were found in stolons and roots than leaves and petiols, because petiols and leaves of *C. asiatica* are more susceptible to chemical compounds during the initial sterilization process of isolation, so the possibility of endophytic fungi to be able to survive during the sterilization process for these two organs tends to be smaller. The strain of endophytic fungi are also the determinant factors for the presence of endophytic fungi in each organ were found in this study. The stolons may have supported higher endophytic abundance, due to their higher biomass providing more site and resource for colonization compare the other organs. The stolons were more favorable for fungal colonization in the *C. asiatica* of Bengkulu accession. In this study stolons was the first organ isolated from endophytic fungi, rarely previous studies isolated of endophytic fungi from stolons because not all plants have stolon. **Phuakjaiphaeo and Kunasakdakul (2015)** studied endophytic fungi from stolons, there were 19 strains from stolons, nine strains from roots, four strains from leaves and four strains from fruits. The differences may be caused by the structure and substrate different between stolons and the other organs, which influence the colonization of fungal endophyte.

According to **Murphy et al. (2013)**, the success of endophytic fungi colonization process is influenced by various factors, including plant tissue types, plant genotypes, types of microorganisms, and environmental conditions (biotic and abiotic factors) (**Hardoim et al., 2015**). The type of colonized plant organ could be influence abundance of endophytic fungi, for example more permanent (strong) plant organs such as roots allow for greater colonization than other organs (**Sun et al., 2011**).

The similar research have been reported in the other endophyte studies, e.i. **Jin et al. (2013)** in *Stellera chamaejasme* L. plants showed colonization rate in the roots reached 52.9%, while in leaves and stems ranged from 46-49%. The root, which have stable environmental conditions for most types of fungi (**Garbeva et al., 2004**), so that endophytic fungi are easier to colonize root organ. **Angelini (2012)** in his research stated that, the root might produce chemical compounds around it, so as to create an environment suitable for the survival of endophytic fungi. The study of **Ghimire et al. (2011)** in elephant grass plants, which showed that the root organs were colonized by 15 ordo of endophytic fungi, while in shoots only 6 ordo and were dominated by soil-borne fungi. The other result was reported by **Mishra et al. (2012)** in several medicinal plants in India. The study stated that the highest colonization rate was found in leaves and the lowest was found in plant roots. The research of **Sun et al. (2011)** in *Bletilla ochracea* (Chinese orchid) plants, the colonization rate of endophytic fungi in stems is higher than in leaves. **Sun et al. (2008)** found that the colonization rate of endophytic fungi from six medicinal plant were higher twig than leaves. According **Rakotoniriana (2007)**, indicated that on the leaves organ of *C. asiatica* from Brazil were found more than the other plant organs.

Diversity index

The overall, the diversity of endophytic fungi in *C. asiatica* plants is $H' = 2.98$. The value of H' is between $1 < H' < 3$ was included in the medium category. Diversity index (H') in each organ are varies (Figure 4). The diversity of endophytic fungi in leaves, roots and stolons are higher than petiols. The highest H' value are found in the leaves organ ($H' = 2.14$), followed by stolon ($H' = 1.79$), and the lowest on the petiol ($H' = 1.1$).

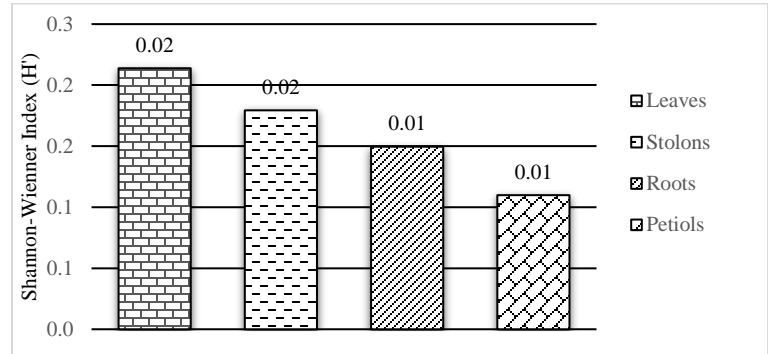


Figure 2 Diversity index of endophytic fungi from *Centella asiatica* of Bengkulu accession

The diversity index (H') of endophytic fungi on leaves of *C. asiatica* had been investigated by **Gupta and Chaturvedi (2017)** previously, and H' obtained which varied in different seasons. The maximum H' value is obtained in the rainy season ($H' = 2.24$), and the lowest is during winter ($H' = 1.7$). Similar results where the leaves have the highest H' compared to other organs are also found in *Bletilla ochracea* plants (**Tao et al., 2012**) and *Cinchona calisaya*, with the value H' on the leaves of $H' = 2.71$ and $H' = 3.0$ (**Radiastuti, 2015**). Leaves have a greater level of diversity. While in other organs such as roots and stolons, the high diversity of endophytic fungi is caused by tissue that is thicker in the roots than the petiols of *C. asiatica*, thus providing room for greater colonization. According to **Radiastuti (2015)**, organs with thicker tissues provide a microenvironment that is suitable for the growth of endophytic fungi, so that it has a greater diversity of endophytic fungi.

The other results were shown in the three herbaceous plants (*Cirsium arvense*, *Plantago lanceolata* & *Rumex acetosa*) (**Wearn et al., 2012**), and on the *Stellera chamaejasme* L. plants ($H' = 2.29$) (**Jin et al., 2013**) have a higher H' value is at the root than the others, while in the *Bauhinia forficata* plant ($H' = 2.206$) it shows that the highest H' value is in the branch (**Bezzera et al., 2015**). Research regarding the diversity of endophytic fungi from plant organs of *C. asiatica* is mostly carried out on the leaf organs. **Ginting et al. (2013)** had been done study of diversity of endophytic fungi on the *C. asiatica* local and Malaysian; **Rakotoniriana (2007)** on *C. asiatica* from Madagascar. While in other organs such as roots, and stolon, it was carried out by **Nalini et al. (2014)**, but there is no mention of the H' value for both organs. The petiol have never been reported previously. Therefore, there are no data H' of endophytic fungi in the petiol organ that can be compared with the results of this study. In this study H' of petiol organ are 1.1 and the lowest H' compare the other organs.

Frequency occurrence

The frequency occurrence of endophytic fungi is calculated to know the distribution of endophytic fungi to *C. asiatica* of Bengkulu accession. Fungal endophyte can be distributed to every organs of *C. asiatica* of Bengkulu accession, with the highest frequency of occurrence of endophytic fungi is located on stolon, meanwhile the lowest occurrence frequency is on the petiol. It is pointed with the amount and the percentage of occurrence frequency of endophytic fungi that appears in every organs.

The highest frequency of occurrence of endophytic fungi was in the stolons (37.7%), followed by roots (32.7%), leaves (19.6%), and petiols (9.8%). *Fusarium oxysporum* 2 was the highest frequency of occurrence in the stolons, with a value of 9.84%, *F. Falciforme* 2 in the roots (9.84%), *Colletotrichum* sp. 2 and *Mycochaetophora gentinae* were the highest in the leaves (3.28%) and *Aspergillus* sp., *Phanerochaete chrysosporium*, *Colletotrichum* sp. 1 were the highest in the petiols (3.28%). *F. Falciforme* 3 and *Phoma multirostrata* were found in the stolons, roots, and leaves. *Colletotrichum* sp. 1 were found in the stolons, leaves and petiols. They weren't included organ specific. The other taxa were distributed in each the stolons, roots, and leaves. There are included specific organ.

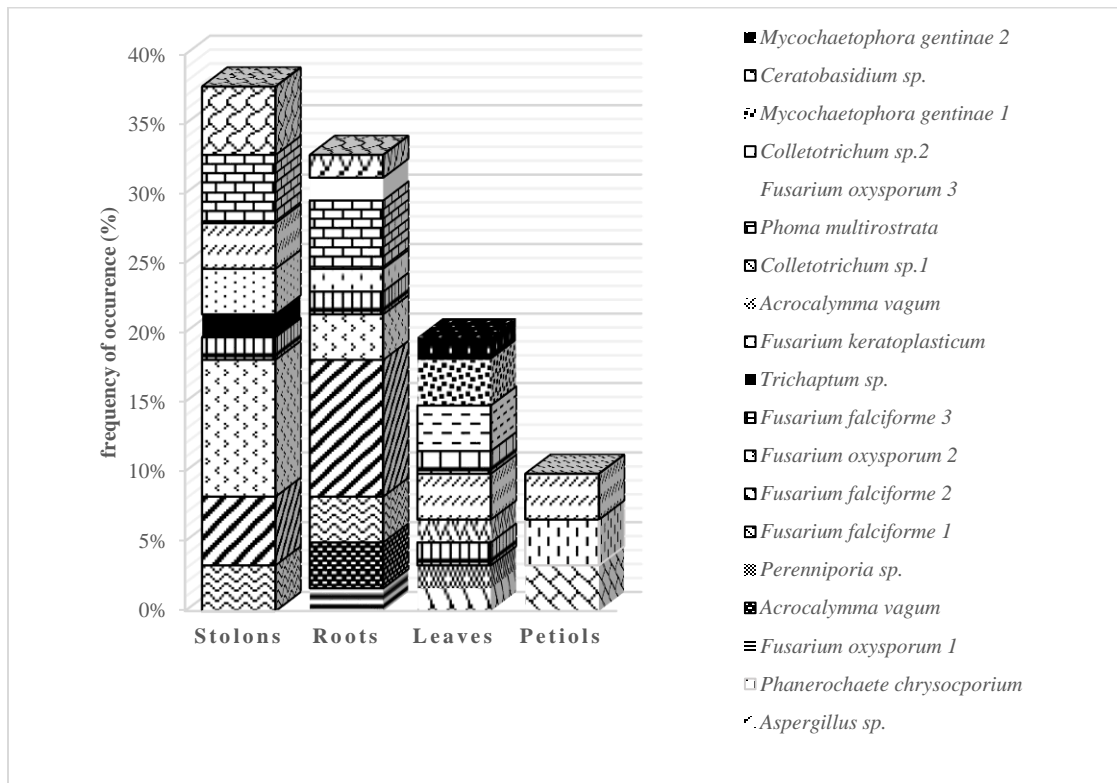


Figure 3 Frequency of occurrence of endophytic fungi from *Centella asiatica* of Bengkulu accession

The distribution of endophytic fungi also affected by the difference of structure and substrate of each host plants organ, as well as the ability of endophytic fungi in the use of the specific substrate. The research result showed that there is no single species found in all organs, but there are several endophytic fungi species that almost found in the all organs of *C. asiatica* of Bengkulu accession, namely *Fusarium solani* 3 and *Phoma multirostrata*. The both species are found in roots, stolons and leaves but not in the petiols.

Other than that it is found there are spesific species that only can be found in the certain organ, namely *Phanerochaete chrysosporium* that only can be found on the petiol, *Acrocalymma vagum*, *Perenniporia* sp., *Colletotrichum* sp. and *Helotiales* sp. only can be found on the leaf organ. This is shows that leaf becomes the organ that colonized the most of the specific endophytic fungi. According to **Shao et al. (2008)**, the amount of endophytic fungi that colonized one type of organ indicates that there is endophytic fungi specificity towards plant tissue.

Endophytic *Fusarium*, *Colletotrichum*, *Phoma* dan *Aspergillus* are known as endophytic fungi that easily can be found on medicinal plants (**Jia et al., 2016**). According **Jin et al. (2013)** that *Fusarium* is one genus of the most found as endophytic fungi. *Phoma* and *Fusarium* are categorized as *Nonclavicipitaceus* that have the ability to widely spread to the plant's organ. Based on grouping criteria of endophytic fungi ecologically according to **Rodriguez et al. (2009)**, *Fusarium* and *Phoma* are categorized as *Nonclavicipitaceus* (NC endophyte) class two. This two class of endophyte has the widest scope of organ plant colonization, because it can colonize sprout, root, branch and leaf. This class also able to spread through seeds to rhizomes, has the highest occurance frequency (90-100%), and able to give positive effect on the health of the host plants which are their habitat or not (**Rodriguez et al., 2009**).

The *Phoma* is known as endophytic fungi that generally could be found colonizing the root organ (**Macia-vicente et al., 2008**) and capable to increase the plant growth by secreting phytohormones in *Cucumis sativus* plants (**Waqas et al., 2012**). Meanwhile, *Fusarium* is known as endophytic because its ability to induce host plants resistance to the pathogen, increasing the health of the host plants in stressful environment (**Bacon and Yates, 2006**). Some of endophytic fungi are found such as *Fusarium* and *Colletotrichum* that are known as pathogens in the plant (**Ya et al., 2010**). This indicates that fungi can be as saprophytic and pathogenic with endophytic phases (**Rosa et al., 2010**). According to **Schulz and Boyle (2005)**, there's no neutral interaction between endophytes and their host, because there are always a balance that allows the defense of endophytic fungi that are antagonistic, when the host defenses limit the development of endophytic fungi themselves.

This research found several fungi that are rarely found as endophytic fungi, namely *Acrocalymma vagum*, *Perenniporia* sp., *Helotiales* sp. and *Trichaptum* sp.

However, there are similarities species of endophytic fungi obtain from tobacco leaves which are *Acrocalymma vagum* (**Jin et al., 2013**), *Acrocalymma aquatica* derived from *Dendrobium houshanense* (**Yi-Min et al., 2015**), *Perenniporia* sp. from *Elaeis guineensis* (**Pinruan et al., 2010**), and *Helotiales* sp. in *Dendrobium officinale* plants (**Chen et al., 2013**).

The dominance level is calculated to determine the type of endophytic fungi to *C. asiatica* plants of accession Bengkulu. It is known that *Fusarium keratoplasticum* *F. oxysporum* dan *Colletotrichum* sp. are dominant species and belong to *Ascomycetes* (Figure 4). The dominance index in the all species dominance are ranged between 0.038-0.01, which means belong to the low category.

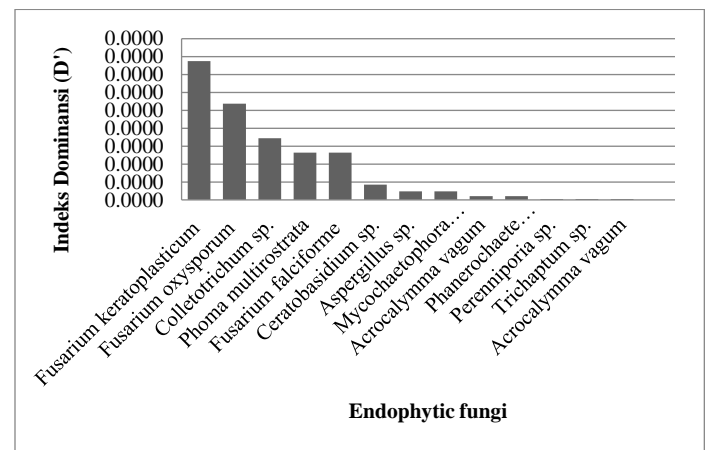


Figure 4 Dominance index of endophytic fungi from *Centella asiatica* of Bengkulu accession

UPGMA analysis

According to UPGMA analysis, with similarity index scored <0.5, the community structure of endophytic fungi divided into 3 clusters (figure 5). Endophytic fungi community in the stolons have similarity with the roots organ (similarity index 0.58). Meanwhile on the leaves and the petiols only 0.2 point, hence both organs were separated into third groups. Similarity index that is close between stolons and roots. It was caused by the 3 dominant species which are *Phoma multirostrata*,

Fusarium solani dan *F. oxysporum*. Additionally, other species present in stolons and roots were almost similar.

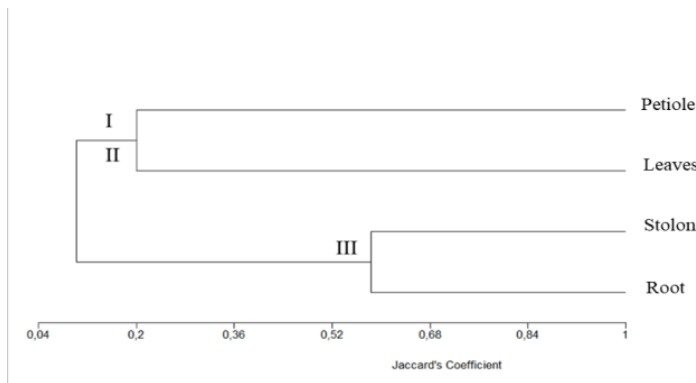


Figure 5 UPGMA Analysis using Jaccard Coefficient

The stolons and roots were separated with leaves and petioles because there was different composition on endophytic fungi in both organs. There were endophytic fungi that only exist on the upper organ (leaves and petioles), however not on stolons and roots. The endophytic fungi were *Phanerochaete chrysosporium* that only can be found on petioles, *Acrocalymma vagum*, *Perenniporia* sp., *Colletotrichum* sp., and *Helotiales* sp. only can be found in leaves organs. The community structure of endophytic fungi on *C. asiatica* of Bengkulu accession were also separated from the petioles because there was only one species of endophytic fungi that is similar between the two organs, namely *Aspergillus versicolor*.

The result of UPGMA analysis with Jaccard coefficient. The research proves that community structure if endophytic fungi in the roots and leaves have low level of similarity. This because the two organs are the surface that interact most dynamically with the environment, dan have differences in terms of water content, nutrient, lighting and UV radiation level (Arnold, 2007; Angelini et al., 2012).

The endophytic fungi arranges the community structure of endophytic fungi in the roots and the stolons have high similarity index value. Almost all fungi arrange the community structure of endophytic fungi on both organs are from *Nonclavicipitaceus* (NC) class 2, which consist of *Phoma*, *Colletotrichum* dan *Fusarium*. This type of in this endophytic fungi usually attacks host plants through lower plants organs such as rhizomes and seeds (Rodriguez et al., 2009). Moreover, the location of the two organs adjacent to the ground, this allows many sources of endophytic fungi inoculums that comes from the soil, commonly known as soil-fungi and mycorrhizae (Domsch et al., 2007).

Meanwhile, similarity index of forming community structure of endophytic fungi on the upper organs (leaves and petioles) are relatively low. Based on the criteria ecology, most endophytic fungi in these two organs are categorizes into *Nonclavicipitaceus* (NC) class 3 includes *Dothidiomycetes*, *Sordariomycetes*, *Eurotiomycetes*, and *Agaricomycetes*. This endophytic group tends to infect the host plants through the upper organs and has an abundant level of diversity (Rodriguez et al., 2009). Class 2 endophytes are also found in conifer plants and tropical plants (Arnold and Lutzoni, 2007). According to Herre et al. (2007), the main source of endophytic fungi propagules in the upper plant organs comes from spores carried by wind and flowing water.

The difference of the community structure of endophytic fungi in various *C. asiatica* of Bengkulu Accession. According to Jia et al. (2016), this dissimilarity is affected by some factors such as environment, genetic background of the host plants, and type of host plants tissue. The environmental condition such as temperature, humidity and availability of soil nutrients are essential factors that determine the type and amount of metabolites produced by the host plants, which indirectly affect the community structure of endophytic fungi (Wu et al., 2013).

CONCLUSION

The endophytic fungi are distributed to the leaves, petioles, stolons and roots of *C. asiatica* of Bengkulu accession and there are 60% segment organ that are not colonized and the highest colonization is in the stolon. The diversity of endophytic fungi in the Pegagan plants (*C. asiatica*) of Bengkulu accession is classified as moderate, and the highest H' is found in leaves organs. The endophytic fungi species that dominate the whole organ of Pegagan plants are *Fusarium solani*, *Fusarium oxysporum* dan *Phoma multirostrata*.

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