Diversity and Antifungal Activity of Endophytic Fungi Associated With Melastoma malabathricum L.

Nor'Aishah binti Hasan1* and Nur Izzati Natasha binti Mohd Sudin1

1School of Biological Sciences, Faculty of Applied Sciences
University of Technology MARA, Negeri Sembilan Branch, Kuala Pilah Campus, 72000 Kuala Pilah, Negeri Sembilan, Malaysia

*Corresponding Author Received: September 10, 2019
E-mail: aishahnib@uitm.edu.my
Accepted: November 23, 2019

Abstract
Medicinal plant has been used in folk medicine and found to harbour endophytes. Endophytes are organisms that colonize internal part of plant tissue without causing any harm to its host where it is producing bioactive compound that can be used for antibacterial and antifungal activity. Endophytic fungi strains (n = 35) were isolated from the leaves, roots, and stems of Melastoma malabathricum from UiTM NS Reserve Forest (Malaysia) to delineate their species composition and potential as biological control agents of C. gloeosporioides anthracnose. Fungal colonization rates of the leaves, roots, and stems were 38.90, 33.33, and 25%, respectively. The isolates were identified as 6 group, belonging to two phyla, Zygomycota (11.20%), and Ascomycota (38.90%) and 4 unidentified group including SSM1 (13.90%), SSM5 (11.11%), SRM3 (13.90%) and SRM4 (8.30%) respectively. Based on the results of dual culture experiments, all isolated endophytic fungi exhibited antifungal activity against C. gloeosporioides anthracnose pathogen, with isolate SSM1 and SSM5 generating significantly differences with broadest inhibition zones with 57.89% and 52.63%, respectively. Our results indicate that the endophytes associated with M. malabathricum could be employed as natural agents controlling C. gloeosporioides anthracnose.

Keywords: antifungal activity, Colletotrichum gloeosporioides, endophytic fungi, Melastoma malabathricum.

INTRODUCTION
Development of resistance by existing pathogenic bacteria and fungi to commercial drugs is relevant problem face by health service and become a serious concern all around the world. This rise due to inappropriate use of antibiotic where a certain individual exploits the finite resources for their own benefits [1]. Therefore, there is an urge to explore an effective antimicrobial and antifungal agent that is low cost and environmentally friendly. Endophytic fungi isolated from medicinal plants can be an alternative to treat disease that causes by pathogenic fungi. Endophyte fungi which originally isolated from their own host plants, seems to produce bioactive compound which may have the potential use in agriculture and medicine [2]. Endophytes fungi are a group of microorganisms that colonize healthy and living tissue of internal plants via quiescent infections without causing any harm or obvious negative effect [3]. Different bio fungicides metabolites such as alkaloids, terpenoids, steroids, phenolics and volatiles isolated and characterized from endophyte fungi show antifungal activity against plant pathogenic fungi [3]. Endophytes are the associations with diverse group of organisms throughout the plant kingdom which provide indirect defense for plants [4]. Among the most important group of eukaryotic organisms which is fungi and very well known for producing various novel metabolites that are directly used as a drugs or functions for various bioactive products. Endophytic represent a wide diversity of microbial adaptation that have successfully evolved in environmental changes, thus it is a valuable of study especially in the field of medical, pharmacology industry and agriculture. Medicinal plants are known to nurture endophytic fungi and related with the pharmaceutical industry [5].

Among medicinal plant, Melastoma malabathricum L. (M. malabathricum) or Senduduk plants that belongs to Melastomataceae family, is widely distributed in south-east Asian region including Malaysia. Generally, the common weeds that freely grow which is Melastomataceae and extensively all over the tropics, mostly in the moist area that can be found in the Indian Ocean Islands, throughout South and South-East Asia, China, Taiwan, Australia, And the South Pacific Ocean [6]. As a medicinal herbal, M. malabathricum has been possess as various medicinal value [6]. Many studies have been reported on isolation of endophytic fungi on M. malabathricum however, a lack of study reported on endophytic study as antifungal activity isolated from M. malabathricum. To explore the ecological importance of the endophytic fungi of M. malabathricum, we explored the distribution and diversity of endophytic fungi associated with this plant and evaluated their biocontrol capacity against pathogenic fungi. To our concern, this is the report describing the antifungal activity of endophytic fungal associated with M. malabathricum that can be used as a natural biocontrol agent against plant pathogenic.

MATERIALS AND METHODS
Collection of plants
Leaves, roots and stems samples of M. malabathricum L. were collected from plants grown at UiTM NS Forest Reserve, Malaysia. For each part, thirty-six samples were collected totaling a sample of one hundred and eight parts which were immediately subjected to endophytic fungi isolates. Immediately after the collection, all samples were sealed in vacuum bags, kept at 4°C, and used within 48h of collection. Authentication of the plant materials was carried out at the Forest Research Institute Malaysia (FRIM).
Isolation of endophytic fungi

Endophytic fungi were isolated from the healthy plants as per the procedure of Kumar et al. [7]. The plant parts (leaf, root and stems) were surface sterilized with 70% ethanol for 2 minutes followed by 1% sodium hypochlorite for 3 minutes. Surface sterilized plant parts were dried on sterile blotting sheet and were cut into 5mm x 5mm segment using a sterile scalpel and 5 replicates were performed for each medium were then placed onto Potato dextrose agar (PDA) supplemented with chloramphenicol 150 mg/l. The Petri dishes were sealed using parafilm and incubated at 28°C. The fungi that grown out from the tissues was isolated and stocked. PDA slants were used to preserve cultures at 4°C for further screening [8].

Identification of endophytic isolates

Morphological features including colony morphology, pigmentation, growth pattern, spore structures, and other hyphal characteristics were used to identify the endophytic fungal isolates with aid of standard mycological manuals [9, 10]. Reproductive spores were examined under microscope. Minimal media were used to grown cultures which failed to produce spores and incubated for several weeks to months.

Colonization frequency analysis (CF)

Frequency of fungal endophytes harbored in plant species were calculated to determine the endophytes richness by the number of segments colonized by endophyte species divided by a total number of segments examined ×100 [11].

\[ \text{Colonization frequency (CF\%)} = \frac{\text{No of individual fungi recorded}}{\text{Total no of segments screened}} \times 100 \]

In vitro evaluation of endophyte fungi against Colletotrichum gloeosporioides

The antagonistic potential of endophytic fungal isolates was assessed through direct confrontation method in accordance to Katoch and Pull [12]. The experiment was performed in triplicate and the mean values were recorded. The C. gloeosporioides pure culture was taken from Mycology and Pathology Laboratory (FRIM). A 5mm agar plug of 7-day old endophyte fungus and pathogen were co-cultured at opposite site in PDA plate. The pathogen alone (without endophyte) was served as control. All the plates were invertedly incubated at 28°C for two weeks. The radial growth of pathogen cultured with/without endophyte fungal was measured daily. Then, the data was transformed into percentage inhibition of radial growth (PIRG) using the formula

\[ \text{PIRG} \% = \frac{\text{CDC}-\text{CDT}}{\text{CDC}} \times 100 \]

Where,

- CDC: radial growth of pathogen colony in the absence of endophyte (measured in cm)
- CDT: radial growth of pathogen colony in the presence of endophyte (measured in cm)

Statistical Analysis

The data obtained from the observation on the fungal colony radial was subjected to analysis of variance (2-way ANOVA). The means were separated by Tukey’s test at p=0.05 with SPSS statistical software.

RESULT AND DISCUSSION

Nowadays, medicinal plants have been exploited for thousand years for medicine and health benefits. Medicinal plants also have been used for thousand years in folk medicines and still are used for their health benefits. It is a natural resource for bioactive compound and habitat for endophytic bacteria and fungi. Nearly, all plant species were found to harbour endophytic. Therefore, the objective of this study was to isolate and identify the endophytic fungi with antifungal activity on M. malabathricum (Figure 1). In total, 36 endophytic fungi isolates were obtained from 108 segments of the leaves, roots and stems. Morphological characteristics were used as a basic identification analysis for the endophytic fungi. The isolates were divided into 6 phenotype groups based on their morphological characteristics.

![Figure 1. Plants selected for the present study.](image-url)
Table 1. List of Endophytes Fungi Growth on Roots, Stems and Leaves of Melastoma malabathricum L.

<table>
<thead>
<tr>
<th>Parts of plant</th>
<th>Total of Plant Segments</th>
<th>Number of Isolated Fungi</th>
<th>Endophyte Fungal Strain</th>
<th>Colonization Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>36</td>
<td>5</td>
<td>SRM3</td>
<td>13.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>SRM2</td>
<td>11.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>SRM4</td>
<td>8.30</td>
</tr>
<tr>
<td>Stem</td>
<td>36</td>
<td>5</td>
<td>SSM1</td>
<td>13.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>SSM5</td>
<td>11.11</td>
</tr>
<tr>
<td>Leaf</td>
<td>36</td>
<td>14</td>
<td>SLM6</td>
<td>38.90</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The morphological identification of fungi isolated was performed on PDA medium for the determination of the macroscopic characteristics of the culture (color and appearance of the fungal colonies). On the other hand, the microscopic observations were made on young crops by determining the morphological characters of seed heads of each fungi based on key identifications of Nasraoui et al. [17].

Observation on SRM2 isolate demonstrates a slow growing fungus which fully grew on a plate after 2 weeks and the colour changed into with age. Microscopic analysis demonstrated that, SRM2 consists of aseptate hyphae and showed unbranching sporangiospores which belong to the phylum Zygomycota SLM6 performed black-greyish colour with white concentric ring and showed rapid growth rate that fully fills the culture plate in four to five days period. The colony also showed a downy and powdery texture. It has septate hyphae and conidiophores which supported by fruiting body of conidiophore. Based on morphological characteristic, this isolate belongs to the phylum Ascomycota. SRM4 and SRM3 isolates exhibited similar growth and morphology with white-yellowish colony in PDA media. However, after one-week growth, a red color colonizing the agar in SRM3 isolate. Both isolates demonstrate aseptate hyphae.

Meanwhile, SSM1 and SSM5 were rapidly growing fungus which occupied the petri plate in a few days. It forms black-greyish colour with brownish colour in the middle part of the colony and crusty texture with black colour of mycelia, respectively. This four endophytic samples remains unidentified because lacking in characterization and information. Although endophytes fungi had major differences between morphology, however, these fungi were difficult to identify at the species level. The use of morphological features was problematic for phylogenetic systematics of hypogenous ascomycetes due to a small set of morphological characteristics and homoplasy [19]. Finding made by Bhardwaj et al. [18] also was reported that some of the isolated endophytic fungi, was not be able to identify as the colony did not show much of its characteristics and sometimes did not produce spores on PDA media. Therefore, phylogenetic analysis based on rDNA sequencing should subsequently employed to identify species that could not be categorized based on their morphological characteristics.

Besides, cultivation on artificial media precludes the isolation of some endophytic fungi, and an accurate, rapid identification technology for these fungi is still needed for some fungal isolates were recalcitrant to culture on the PDA medium.
Six isolated endophytic fungi from *Melastoma malabathricum* were proceeded with antifungal activity against *C. gloeosporioides*, causal organism that caused Anthracnose where it is the most widespread and serious postharvest disease that affect tropical fruits such as mango, papaya and avocado [20]. Antifungal or antagonistic activity were assessed by dual culture method in terms of percent inhibition radial growth (PIRG %). Antagonistic study was observed for two weeks to obtain the growth radius (cm) of pathogenic fungi. For dual culture method, each of the endophyte fungi isolates and pathogenic fungi were ensured to has the same age of 7 days old and same sized agar disc by using same sized of cork-borer.

Result in this study showed that all 6 isolated endophytes fungal from *Melastoma malabathricum* L. showed an antifungal
activity with a significance difference (p<0.05) against *C. gloeosporioides* (Figure 3). All the isolated in this experiment were effective and positives in inhibiting the growth of all *C. gloeosporioides* isolates range from 42% until 58%. The most effective antifungal activity was the SSM1 with highest antifungal activity against *C. gloeosporioides* with 57.89% of inhibition radial growth by using dual culture method (Table 2). Three isolated endophytes fungus from *Melastoma malabathricum* (SRM2, SRM3 and SLM6) was found with similar activity of inhibition zone against *C. gloeosporioides* with 47.37%. The lowest activity against *C. gloeosporioides* with 42.11% was SRM4.

**Table 2.** Antifungal activity of isolated endophyte fungi using dual culture method against pathogenic fungi, *Colletotrichum gloeosporioides*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Percent inhibition radial growth against *C. gloeosporioides (%) Dual culture assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSM1</td>
<td>57.89 ± 0.06d</td>
</tr>
<tr>
<td>SRM2</td>
<td>47.37 ± 0.15b</td>
</tr>
<tr>
<td>SRM3</td>
<td>45.53 ± 0.06i</td>
</tr>
<tr>
<td>SRM4</td>
<td>42.11 ± 0.21d</td>
</tr>
<tr>
<td>SSM5</td>
<td>52.63 ± 0.05e</td>
</tr>
<tr>
<td>SLM6</td>
<td>47.37 ± 0.10f</td>
</tr>
</tbody>
</table>

*Mean ± SD with different letter showed significantly differences (p<0.05) among isolates within column.*

For our concern, this is the first study reported the result for antifungal activity of endophytic fungi against *Colletotrichum gloeosporioides* from *Melastoma malabathricum*. However, there is a study reported on the antibacterial activity of endophyptic fungal made by Mishra 2016 against 4 different types of bacteria, *Enterococcus hirae, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*. There are a few studies reported the antifungal activity on endophytic fungi with different plant sample which Bonatelli [21] stated that Pestalotiopsis sp with lowest antifungal activity against *Colletotrichum gloeosporioides* by using in vitro dual assay. This proved that endophytes fungi have a key role in controlling plant pathogenic fungi [21].

**CONCLUSION**

The results of this study demonstrate the great antifungal potential of endophytic fungi isolated from *M. malabathricum* against pathogenic fungi. Therefore, it suggests that these endophytes can be important sources of bioactive substances which may useful for new drug discovery.

**ACKNOWLEDGEMENT**

The work was performed at UiTM Cawangan Negeri Sembilan. Author would like to thank UiTM for providing all the necessary facilities for the successful completion of the research work.

**REFERENCES**


