

9-30-2019

## Fungal Community of Culturable Fungal Endophytes Associated with *Cinchona calisaya* Collected from Gambung, West Java, Indonesia

Iman Hidayat

*Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Bogor 16911, Indonesia, imanhidayat@yahoo.com*

Nani Radiastuti

*Faculty of Science and Technology, Syarif Hidayatullah State Islamic University, Banten 15412, Indonesia*

Gayuh Rahayu

*Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Bogor 16680, Indonesia*

Izumi Okane

*Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8577, Japan*

Follow this and additional works at: <https://scholarhub.ui.ac.id/science>

---

### Recommended Citation

Hidayat, Iman; Radiastuti, Nani; Rahayu, Gayuh; and Okane, Izumi (2019) "Fungal Community of Culturable Fungal Endophytes Associated with *Cinchona calisaya* Collected from Gambung, West Java, Indonesia," *Makara Journal of Science*: Vol. 23 : Iss. 3 , Article 1.

DOI: 10.7454/mss.v23i3.11258

Available at: <https://scholarhub.ui.ac.id/science/vol23/iss3/1>

This Article is brought to you for free and open access by the Universitas Indonesia at UI Scholars Hub. It has been accepted for inclusion in Makara Journal of Science by an authorized editor of UI Scholars Hub.

## Fungal Community of Culturable Fungal Endophytes Associated with *Cinchona calisaya* Collected from Gambung, West Java, Indonesia

Iman Hidayat<sup>1\*</sup>, Nani Radiastuti<sup>2</sup>, Gayuh Rahayu<sup>3</sup>, and Izumi Okane<sup>4</sup>

1. Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Bogor 16911, Indonesia

2. Faculty of Science and Technology, Syarif Hidayatullah State Islamic University, Banten 15412, Indonesia

3. Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Bogor 16680, Indonesia

4. Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8577, Japan

\*E-mail: imanhidayat@yahoo.com

Received March 20, 2019 | Accepted August 7, 2019

### Abstract

Previous studies regarding endophytic fungi associated with *Cinchona* were focused on the analysis of quinine and related fungal metabolites. In the current study, the community structure of culturable endophytic fungi associated with *C. calisaya* (i.e., leaf, petiole, twig, root, flower, and fruit) were analyzed and elucidated. Representative isolates from various morphotypes were identified based on a sequence generated from the Internal Transcribed Spacer (ITS) rDNA region. A total of 638 fungal strains were isolated from 700 plant segments. All isolates belong to the phylum Ascomycota and are dominated by a member of Sordariomycetes. The fungal endophyte community within *C. calisaya* consists of 23 known species, 10 species complexes, and 6 unidentified species. Based on the Shannon-Wiener diversity index, the leaves and fruits host the most diverse endophytic fungi, followed by twigs, petioles, and bark. Members of the genus *Diaporthe* are commonly found in all *C. calisaya* organs. *Neofussicoccum* sp. was only one found within twigs; the *Colletotrichum gloeosporioides* complex and *Guignardia mangiferae* were only found within leaves; the *C. brasiliense* complex was detected in flowers; and *Fusarium oxysporum* is specific to roots.

**Keywords:** community, endophyte, fungi, ITS, quina

### Introduction

Endophytes are microbes that asymptotically inhabit internal plant tissues for at least one part of their life cycle without causing apparent harm to their host [1]. Higher endophyte diversity is expected to be found in tropical plants [2]. Among them, endophytic fungi widely distributed within plant tissues are rich in species diversity [3]; these fungi are usually found in all major plant lineages, including trees, shrubs, grasses, and ferns [4]. They colonize both the vegetative (i.e., leaves, petioles, bark, or stems) and reproductive tissues of their hosts [5-6].

Due to a growing population in many quina plantation areas in Java, as well as changes in priorities for industrial plants implemented by the government, quina plantation areas have been drastically reduced. Therefore, it has become necessary to discover quinine production alternatives, among them, quina-associated fungal endophytes are promising agents [7-11].

Information concerning the fungal endophyte community, such as its diversity, species dominance, and their distribution within different *C. calisaya* organs, is important for understanding their roles in relation to quinine and the production of related metabolites of their hosts. Stierle *et al.* [12] noted that fungal endophytes are known for their potential to produce the same bioactive compounds as their host, i.e., anticancer taxol produced by *Pestalotiopsis microspora* isolated from *Taxomyces andreanae*. Radiastuti *et al.* [13] also reported that of a total of 10 genera of endophytic fungi from quina that can produce quinine, only members of *Diaporthe* and *Fusarium* isolated from twigs produced a high concentration of quinine. Both are dominant taxa associated with quinine. The highest concentration of quinine was produced by *Diaporthe* isolated from twigs.

However, in studies of endophytic fungi associated with *Cinchona* spp. (quina), most were primarily focused on fungal isolation and screening of their secondary metabolites. Some of these endophytic fungi were isolated

from bark [7-9]. To date, there have been no studies examining the fungal community within all the quina organs. In this study, a culture-based method was selected for studying fungal community structure (i.e., diversity, species dominance, and fungal distribution in different plant tissues), and this method can provide genetic resource materials for secondary metabolite biotechnology.

## Materials and Methods

Flowers, leaves, petioles, stems, bark, and roots of *C. calisaya* were collected from the quina plantation managed by the Research Center for Tea and Quina in Gambung, West Java, Indonesia in September 2012. The specimen trees are located at 7°8'35.78"S, 107°30'59.55"E, and 1400 m above sea level. Five segments of each healthy organ were collected from five individual plants and stored in zippered plastic bags. The collection bags were sealed and labeled with the name of the host, collection site, date, and collector(s). All specimens were stored under cold conditions in an ice box prior to isolation.

Fungal endophytes were isolated as previously described [14] with some modifications. The samples were washed with tap water, then surface-sterilized using 70% ethanol for 1 min, followed by soaking in a 3% sodium hypochlorite solution for 2 min, then 70% ethanol for 20 s. The samples were rinsed three additional times in sterile distilled water followed by drying in sterilized tissue paper for at least 6 h. A total of 100 µL of water from the final wash was poured onto agar medium as a control. After drying, samples were cut into 1 × 2 cm segments and placed on the surface of Malt Extract Agar (Difco, USA) medium (4 segments/dish). All dishes were incubated at room temperature, and the growth of endophytic fungal mycelium was observed every day for 30 d. This procedure was conducted in three replicates for each sample. The growing colonies were purified using a hyphal tip isolation method to obtain pure cultures, which were preserved at the Bogor Agriculture University Culture Collection (IPBCC) [13].

All isolated fungi were grouped into different morphotypes based on their colony characteristics, i.e., color on agar surface and reverse, elevation, texture, type of mycelium, and margin shape. Representative cultures from each morphotype were selected for molecular analysis. Genomic DNA from fungal mycelia were extracted using the Phytopure™ DNA extraction kit (GE Healthcare, UK) following the manufacturer's protocol. Amplification was performed using polymerase chain reaction (PCR) in a 25 µL total volume as follows: 10 µL nuclease free water, 12.5 µL DreamTaq® green master mix (Thermo Fisher Scientific, USA), 0.5 µL of each forward and reverse primer, 0.5 µL DMSO, and 1 µL DNA template. The primer

pair consisting of ITS5 (forward: 5'-TCCTCCGCTTATTGATATGC-3') and ITS4 (reverse: 5'-TCCGTAGGTGAACCTGCGC-3') [15] were used to amplify the ITS region, encompassing 5.8S rDNA. The PCR conditions were set as follows: 90 s at 95 °C for initial denaturation, followed by 35 cycles of 95 °C for 30 s for denaturation, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s, followed by 5 min at 72 °C for final extension. All PCR reactions were conducted using a T100 thermal cycler (Bio-Rad, USA).

PCR products were electrophoresed on a 1% (w/v) agarose gel in 1x TAE buffer at 100 V for 30 min. The gel was soaked in an ethidium bromide solution for 30 min prior to UV light examination using the Gel Doc XR system (Bio-Rad, USA). Purified PCR products were sent to 1stBASE (Malaysia) for sequencing. Nucleotide sequences obtained from the respective primer pairs (ITS5 and ITS4) were examined and edited by direct examination using Chromas Pro 1.41 software (Technelysium Pty Ltd., Australia). Species names from the acquired nucleotide sequences were obtained by finding the most homologous sequence through BLAST (The Basic Local Alignment Search Tool) analysis conducted in MEGA 7 [16].

Community structure was expressed as the diversity index, colonization rate, and frequency of occurrence of fungal species within each plant tissue. Each fungal colony was considered as an individual fungus. The Shannon-Wiener diversity index ( $H'$ ) was employed to evaluate and compare the diversity of fungal communities between different *C. calisaya* organs.  $H'$  was calculated according to the following formula:

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

where  $k$  is the total number of fungal species in the community, and  $p_i$  is the proportional contribution of species  $i$  to the total number of individuals [17].

The colonization rate (%) was calculated as the percentage of the total number of segments colonized by fungi divided by the total number of segments observed, while the frequency of occurrence (FO) of endophytic species was calculated in order to estimate species dominance and fungal distribution. Fungal colonization and occurrence within different organs were calculated using the following formula:

Frequency of occurrence of species  $i$  (F) = (Number of strains in species  $i$ /Total number of strains found) × 100%.

Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis was performed using Jaccard's coefficient with the Multi-Variate Statistical

Package software version 3.13r (Kovach Computing Services) on the bases of the diversity index, presence of fungi, and frequency of fungal occurrence. A dendrogram of community relatedness was reconstructed on the basis of similarity distance as calculated by Jaccard's coefficient.

### Results and Discussion

*Cinchona* spp. is the main source of quinine production in Indonesia. However, the reduction of quina plantations, along with delayed regeneration of available *Cinchona* plantation areas, has limited quinine production in this region. Studies concerning biodiversity of endophytic fungi associated with *C. calisaya* and their related quinine generation potential could provide an alternative solution for quinine production.

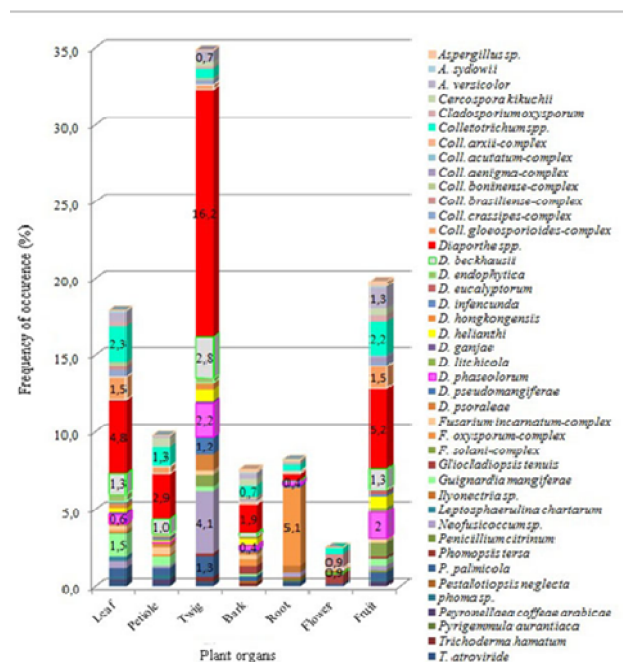
In this study, a total of 638 fungal isolates were obtained from 700 segments derived from *C. calisaya* tissues. The fungal cultures were grouped into 39 morphotypes [13]. BLAST analysis of the ITS sequences showed that these 39 morphotypes primarily belonged to the class Sordariomycetes (78.2%), followed by Dothidiomycetes (14.1%) and Eurotiomycetes (7.7%). Of them, 23 morphotypes were determined to the species level, 10 morphotypes belonged to species complexes, and the remaining six morphotypes were unidentified (Table 1). This obtained number of fungal strains was higher than expected, as previous similar studies on fungal endophytes associated with *C. calisaya* did not report high diversity of fungal taxa [7-11, 18]. In addition, 39 of the endophytic fungal species taxa derived from *Cinchona* are reported here as follows (Table 1): *Aspergillus* sp., *A. sydowii*, *A. versicolor*, *Penicillium citrinum*, *Cercospora kikuchii*, *Cladosporium oxysporum*, *Guignardia mangiferae*, *Leptosphaerulina chartarum*, *Neofusicoccum* sp., *Phoma* sp., *Peyronellaea coffeae-arabicae*, *Colletotrichum acutatum* complex, *C. aenigma* complex, *C. arxii* complex, *C. boninense* complex, *C. brasiliense* complex, *C. crassipes* complex, *C. gloeosporioides* complex, *Diaporthe* sp., *D. beckhausii*, *D. endophytica*, *D. eucalyptorum*, *D. infecunda*, *D. hongkongensis*, *D. litchicola*, *D. phaseolorum*, *D. pseudomangiferae*, *D. psoraleae*, *F. incarnatum* complex, *F. oxysporum* complex, *F. solani* complex, *Gliocladiopsis* sp., *Ilyonectria* sp., *Pestalotiopsis neglecta*, *Phomopsis palmicola*, *P. tersa*, *Pyrigemmula aurantiaca*, *Trichoderma hamatum*, and *T. viride*. This study also confirms the findings of several studies reporting that most fungal endophytes belong to Ascomycota [6, 19-20].

For the distribution of fungal endophytes within different plant organs, members of *Diaporthe* spp. exhibited the widest distribution (Figure 1) and were found predominantly within twigs (16.2%). Within the fruit, leaf, petiole, bark, root, and flower, the FO of *Diaporthe* spp.

were 5.2%, 4.8%, 2.9%, 1.9%, 0.4%, and 0.1%, respectively. *Neofusicoccum* sp. was also a common fungal endophyte found in twigs (FO = 4.1%). The *C. gloeosporioides* complex and *G. mangiferae* (FO = 1.5%) were the most common taxa appearing in leaves,

**Table 1. List of the Identified Endophytic Ascomycota Isolates from *C. calisaya* Based on the ITS rDNA Region**

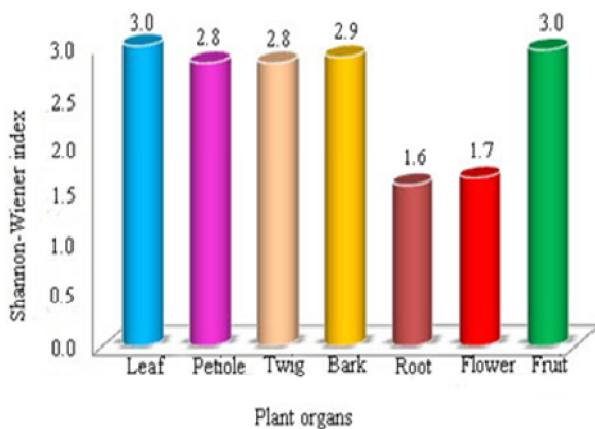
Class	Species name
Eurotiomycetes	<i>Aspergillus</i> sp., <i>A. sydowii</i> , <i>A. versicolor</i> , <i>Penicillium citrinum</i>
	<i>Cercospora kikuchii</i> , <i>Cladosporium oxysporum</i> , <i>Guignardia mangiferae</i> ,
Dothidiomycetes	<i>Leptosphaerulina chartarum</i> , <i>Neofusicoccum</i> sp., <i>Phoma</i> sp., <i>Peyronellaea coffeae-arabicae</i>
	<i>Colletotrichum acutatum</i> complex, <i>C. aenigma</i> complex, <i>C. arxii</i> complex, <i>C. boninense</i> complex, <i>C. brasiliense</i> complex, <i>C. crassipes</i> complex, <i>C. gloeosporioides</i> complex, <i>Diaporthe</i> sp., <i>D. beckhausii</i> , <i>D. endophytica</i> , <i>D. eucalyptorum</i> , <i>D. infecunda</i> , <i>D. hongkongensis</i> , <i>D. litchicola</i> , <i>D. phaseolorum</i> , <i>D. pseudomangiferae</i> , <i>D. psoraleae</i> , <i>Fusarium incarnatum</i> complex, <i>F. oxysporum</i> complex, <i>F. solani</i> complex, <i>Gliocladiopsis</i> sp., <i>Ilyonectria</i> sp., <i>Pestalotiopsis neglecta</i> , <i>Phomopsis palmicola</i> , <i>P. tersa</i> , <i>Pyrigemmula aurantiaca</i> , <i>Trichoderma hamatum</i> , <i>T. viride</i>
Sordariomycetes	



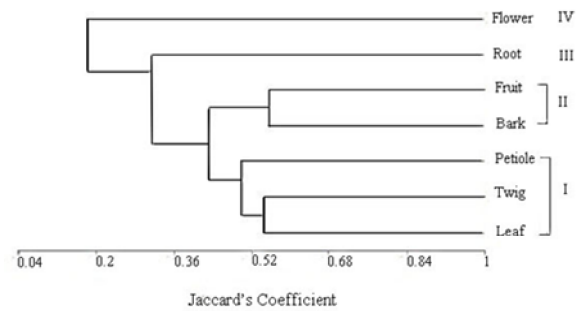
**Figure 1. Frequency of Occurrence (FO) of Fungal Endophytes in Different *C. calisaya* Organs**

the *C. brasiliense* complex was the most common taxon in flowers (FO = 0.9%), and *F. oxysporum* (FO = 5.1%) was the most common fungal endophyte in roots. This study showed that members of *Diaporthe* spp. and *Colletotrichum* spp. were the dominant fungal endophytes in *C. calisaya*. The prevalence of *Diaporthe* spp. and *Colletotrichum* spp. within different hosts were reported by Kumaresan and Suryanarayanan [21] as well as Douanla-Meli and Langer [22]. In another study, Maehara *et al.* [9] also showed that *Diaporthe* spp. along with their asexual stage (*Phomopsis* spp.) were dominant in *C. ledgeriana*. Based on a comprehensive analysis of *Diaporthe* species phylogenetics from different hosts, Gomes [23] noted that members of *Diaporthe* have commonly been discovered as endophytes in nearly all plants.

The Shannon-Wiener diversity index ( $H'$ ) is commonly applied as a measurement of fungal diversity [17, 24-26]. This index showed that leaves ( $H' = 3.0$ ) and fruits ( $H' = 3.0$ ) exhibited the most diverse endophytic fungi, followed by bark ( $H' = 2.9$ ), twigs ( $H' = 2.8$ ), and petioles ( $H' = 2.8$ ). Flowers ( $H' = 1.7$ ) and roots ( $H' = 1.6$ ) harbored less diverse endophytic fungi (Figure 2). This indicates that although fungal endophytes occur in all plant tissues, their diversity varies within each organ. This is probably due to variations in tissue type and nutrient availability affecting fungal diversity within organ colonization [26, 27]. Tao *et al.* [17] also reported that the highest fungal diversity occurred in the leaves of *Bletilla ochracea*. The score on the Shannon-Wiener index increases either by containing additional unique species or by having greater species uniformness [28]. Unique species were also found in the leaves and fruit of *L. chartarum* and *P. citrinum*, respectively. Tao *et al.* [17] also reported that the diversity of endophytic fungi within the leaves of *Bletilla ochracea* was higher than that within the roots.



**Figure 2. The Shannon-Wiener Diversity Index ( $H'$ ) of Fungal Endophytes in Different *C. calisaya* Organs**



**Figure 3. UPGMA Analysis Elucidated the Relationship Among Fungal Endophytes Communities in Different *C. calisaya* Organs**

The UPGMA analysis of fungal community structure in *C. calisaya* found four distinct clusters of fungal communities (Fig. 3). The fungal community in twigs was more closely related to those in the leaf and petiole (similarity index >0.52). The fungal community in bark was similar to that within the fruit, while those in the root and flower formed distinct communities. The close similarity of fungal endophytic communities from the leaf, petiole, and twig were possibly due to the presence of two dominant species, i.e., *C. gloeosporioides* complex and *Diaporthe* spp. The flower and root fungal communities were apparently segregated due to differences in fungal endophyte composition.

**Conclusion**

Analysis of the fungal endophyte community structure associated with *C. calisaya* found that members of *Diaporthe* and *Colletotrichum* were the dominant species. The most highly diverse fungal endophytic community associated with *C. calisaya* was found within leaves and fruits.

**Acknowledgments**

This study was supported by Science and Technology Research Partnership for Sustainable Development (SATREPS) which is a research program in collaborated with the Japan Science and Technology Agency (JST), the Japan International Cooperation Agency (JICA), and Research Center for Biology, the Indonesian Institute of Sciences (LIPI) (2011-2015). We also thank the Tea and Cinchona Plantation Research Center in Gambung, West Java, Indonesia for sampling.

**References**

[1] Petrini, O. 1991. Fungal endophytes of tree leaves. In Andrews, J.H., Hirano, S.S. (eds.), Microbial ecology of leaves. New York, Springer Verlag. pp.179-197.

- [2] Banerjee, D. 2011. Endophytic fungal diversity in tropical and subtropical plants. *J. Microbiol.* 6(1): 54-62. doi: 10.3923/jm.2011.54.62.
- [3] Qiu, S.J., Dong, G.L., Wei, Z., Xiang, P.W., Fu, C.D. 2008. Diversity and ecological distribution of endophytic fungi associated with medical plants. *Sci. China Ser. C-life Sci.* 51(8): 751-759. doi: 10.1007/S11427-008-0091-2.
- [4] Arnold, A.E., Maynard, Z., Gilbert, G.S. 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. *Mycol. Res.* 105(12): 1502-1507. doi: 10.1017/S0953756201004956.
- [5] Arnold, A.E., Herre, E.A. 2003. Canopy cover and leaf age affect colonization by tropical fungal endophytes. *Mycologia.* 95(3): 388-398.
- [6] Arnold, A.E. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol. Rev.* 21(2-3): 51-66, doi: 10.1016/j.fbr.2007.05.003
- [7] Simanjuntak, P., Parwati, T., Bustanussalam, Prana, T.K., Wibowo, S., Shibuya, H. 2002. Isolasi dan kultivasi mikroba endofit penghasil senyawa alkaloid kinkona dari *Cinchona* spp. *J. Mikrobiol. Indo.* 7(2): 27-30.
- [8] Winarno, E.K. 2006. Produksi alkaloid oleh mikroba endofit yang diisolasi dari batang kina *Cinchona ledgeriana* Moens dan *Cinchona pubescens* Vahl (Rubiaceae). *J. Kim. Ind.* 1: 59-66.
- [9] Maehara, S., Simanjuntak, P., Kazuyoshi, O., Hirotaka, S. 2010. Composition of endophytic fungi living in *Cinchona ledgeriana* (Rubiaceae). *J. Nat. Med.* 64(2): 227-230. doi: 10.1007/s11418-009-0380-2.
- [10] Maehara, S., Simanjuntak, P., Chinami, K., Kazuyoshi, O., Hirotaka, S. 2011. *Cinchona* alkaloid are also produced by an endophytic filamentous fungus living in *Cinchona* plant. *Chem. Pharm. Bull.* 59(8): 1073-1074, doi: 10.1007/s11418-009-0380-2.
- [11] Shibuya, H., Kitamura, C., Maehara, S., Nagahata, M., Winarno, H., Simanjuntak, P., Kim, H.S., Wataya, Y., Ohashi, K. 2003. Transformation of *Cinchona* alkaloids into 1-N-Oxide derivatives by endophytic *Xylaria* sp. isolated from *Cinchona pubescens*. *Chem. Pharm. Bull.* 51(1): 71-74.
- [12] Stierle, A., Strobel, G.A., Stierle, D. 1993. Taxol and taxane production by *Taxomyces andreanae*. *Sci.* 260: 214-216. doi: 10.1126/science.8097061.
- [13] Radiastuti, N. 2015. Diversity of culturable endophytic fungi in *Cinchona calisaya* Wedd.: molecular phylogeny and alkaloid profile. Dissertation. Graduate Programme. Biology Department, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Dramaga.
- [14] Mostert, L., Crous, P.W., Kang, J.C., Philips, A.J.L. 2001. Species of *Phomopsis* and *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. *Mycologia.* 93(1): 146-167.
- [15] White, T.J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds.), *PCR protocols: a guide to methods and applications*. Academic Press, San Diego. pp. 315-322.
- [16] Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33(7): 1870-1874.
- [17] Tao, G., Liu, Z., Sun, B., Zhu, Y., Cai, L., Liu, X. 2012. Occurrence and diversity of endophytic fungi in *Bletilla ochracea* (Orchidaceae). *Afr. J. Microbiol. Res.* 6: 2859-2868. doi: 10.5897/AJMR11.1116.
- [18] Mumpuni, A., Purwati, T., Simanjuntak, P. 2004. Produksi asam lemak oleat oleh mikroba endofit *Sporodibolus salmonicolor* dari tumbuhan kina (*Cinchona pubescens* Vahl.). *Alchemy.* 3: 16-21.
- [19] Davis, E.C., Franklin, J.B., Shaw, A.J., Vilgalys, R. 2003. Endophytic *Xylaria* (Xylariaceae) among Liverworts and angiosperms: phylogenetics, distribution, and symbiosis. *Am. J. Bot.* 90: 1661-1666.
- [20] Khan, R., Shahzad, S., Choudhary, M.I., Khan, S.A., Ahmad, A. 2010. Communities of endophytic fungi in medical plant *Withania Somnifera*. *Pak. J. Bot.* 42(2): 1281-1287.
- [21] Kumaresan, V., Suryanarayanan, T.S. 2002. Endophyte assemblages in young, mature and senescent leaves of *Rhizophora apiculata*, evidence for the role of endophytes in mangrove litter degradation. *Fungal Divers.* 9: 81-91.
- [22] Douanla-Meli, C., Langer, E. 2012. Diversity and molecular phylogeny of fungal endophytes associated with *Diospyros crassiflora*. *Mycology.* 3(3): 175-187. doi: 10.1080/21501203.2012.705348.
- [23] Gomes, R.R., Glienke, C., Videira, S.I.R., Lombard, L., Groenewald, J.Z., Crous, P.W. 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia.* 31: 1-41. doi: 10.3767/003158513x666844.
- [24] Kumaresan, V., Suryanarayanan, T.S. 2001. Occurrence and distribution of endophytic fungi in a mangrove community. *Mycol. Res.* 105: 1388-1391. doi: 10.1017/S0953756201004841.
- [25] Kumar, D.S.S., Hyde, K.D. 2004. Biodiversity and tissue-recurrence of endophytic fungi in *Tripterium wilfordii*. *Fungal Divers.* 17: 69-90.
- [26] Wearn, J.A., Sutton, B.C., Morley, N.J., Gange, A.C. 2012. Species and organ specificity of fungal endophytes in herbaceous grassland plants. *J. Ecol.* 100(5): 1085-1092. doi: 10.1111/j.1365-2745.2012.01997.x

[27] Wang, Y., Guo, L.D. 2007. A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis*. *Cad. J. Bot.* 85(10): 911-917.

[28] Banerjee, D., Mahapatra, S., Manna, S., Mukherjee, R., Mukherjee, S., Pati, B.R. 2006. Occurrence of Endophytic fungi in *Vitex nugendo*. *J. Bot. Soc. Bengal.* 60: 28-31.