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Short Communication: Microfungal diversity on leaves of *Eusideroxylon zwageri*, a threatened plant species in Sarawak, Northern Borneo

A. LATEEF ADEBOLA[1,2,♀], MUID SEPIAH[1], MOHAMAD H. BOLHASSAN[1], MANSOR WAN ZAMIR[1]

[1] Department of Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan-94300, Sarawak, Malaysia. Tel./fax.: +60-146902928, ♥email: lateef.aa@unilorin.edu.ng

[2] Department of Plant Biology, Faculty of Life Science, University of Ilorin, Nigeria

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Abstract. *Adebola AL, Sepiah M, Bolhassan MH, Wan Zamir M. 2015. Microfungal diversity on leaves of* Eusideroxylon zwageri, *a threatened plant species in Sarawak, Northern Borneo. Biodiversitas 16: 264-268.* A survey of the microfungal communities on green leaves and leaf litters of an endangered plant species, *Eusideroxylon zwageri* Teijsm. & Binn. (belian) was carried out for the first time. A total of 200 leaf segments were plated on both water agar and malt extract agar. 74 fungal species were identified from both leaf types with more fungal taxa found on the green leaves, with a Shannon diversity index of 3.85 compared to that on litters, 2.63 and the similarity between the microfungal communities on both leaf types was low with a Bray-Curtis similarity index of 0.366. The most dominant species on both leaf types includes *Aphanocladium areanarum, Trichoderma koningii, Nectria* sp., *Chalara pteridina,* Hyphomycetes sp.3, hyaline *Mycelia sterilia, Circinotrichum* sp., *Phoma* sp., *Acremonium macroclavatum, Chaetopsina* sp., *Physarum* sp., *Beltrania rhombica* and *Colletotrichum acutatum*.

Keywords: Endophytic, green leaves, leaf litters, new record, saprophytic

INTRODUCTION

General knowledge on the microfungal diversity and distribution is still inadequately understood. More studies have been done on fungal diversity and their spatial distribution in the temperate regions as compared to the tropics (Hawksworth 2001; Hawksworth and Rossman 1997). Many areas and substrates still remain unstudied in the world, most especially in the tropics and same applies to many plant species which are not yet studied for their associated microfungal communities. The most accepted fungal estimate of 1.5 million by Hawksworth (1991, 2001) was considered as too small by some authors (Cannon 1997; O'Brien et al. 2005) on the basis that the used plant to fungus ratio of 1:6 used by Hawksworth, which assumed the plant diversity as 270,000, was too low, pointing out that there are about 300,000-320,000 plant species (Prance et al. 2000), 420,000 spp. (Govaerts 2001) and 117,734-575,320 spp. (Wortley and Scotland 2004). An important area of global fungal diversity which has been often overlooked is microfungi on vulnerable, threatened and endangered plant species. There is a wide gap of data on microfungi associated with many rare plant species, in terms of host-specific fungi and also fungal disease caused to the plants (Buchanan et al. 2002).

Eusideroxylon zwageri Teijsm. & Binn. (belian tree), is a typical case study of unstudied rare plants. *E. zwageri* is the only accepted species in the genus *Eusideroxylon* which belongs to the family Lauraceae. This plant species, also called the Borneo Ironwood, is native to the Southeast Asian forest and has been listed in the IUCN Global Red List of Threatened Species as an endangered species due to over logging and habitat destruction (IUCN 1998). To the best of our knowledge, no studies have been found in literature on microfungal communities on belian tree. At the plant family level (Lauraceae), comparatively few studies have been done on plant species belonging to the family Lauraceae, an example is the study done by (Paulus et al. 2006) on *Cryptocarya mackinnoniana* from which 81 fungal taxa were identified using direct observation method and on *Chlorocardium rodiei* by Cannon and Simmons (2002) in which only 10 endophytic fungi were identified.

This study aims at revealing the microfungal communities on green leaves and leaf litters of *E. zwageri* (belian tree) from Kubah National Park in Sarawak, Malaysia. The implications of this study will be far reaching in the understanding, protection and conservation of the belian tree.

MATERIALS AND METHODS

Sampling

Green leaves and leaf litters were collected in March, 2014 from under a belian tree (N 01°36'760, E 110°11'794), 127 m above sea level, at the base of the camp in Kubah National Park in Sarawak, Malaysia. In this Park, this is the only known location of belian tree and this area has a very rough terrain. Coupled with this, collection of belian samples is restricted by the Park officials. Leaf

samples were collected randomly under the belian tree, green leaves as just fallen green leaves and litters as already brown and weak leaves. The belian trees were very tall and it is practically impossible to detach a leaf from it. The samples collected were put in plastic bags, labeled and transported to the laboratory for processing.

Isolation of microfungi

Isolation of microfungi was based on the methods of Rakotoniriana et al. (2008) and Lateef et al. (2014) for endophytic fungi and saprobic fungi respectively, with some modifications. For endophytic fungi, the leaves were washed under running tap water to remove dust and debris adhering to them. The leaves were then cut into 1 cm^2 with and without the midribs under aseptic conditions using a sterile scalpel. They were then surface sterilized with 70% ethanol for one minute, then in 10 % hydrogen peroxide (H_2O_2) solution for five minutes, rinsed with 70% ethanol for one minute and finally rinsed with deionized sterile distilled water five times to remove the sterilants and blotted on sterile filter paper to remove excess water. Five segments were plated separately on water agar (WA) and malt extract agar (MEA). The Petri dishes were sealed with parafilm and incubated at room temperature. Observation and isolation of the growing microfungi starts from the third day of incubation for MEA and four weeks for WA.

For saprophytic microfungi, the leaf samples were washed with double sterilized distilled water, cut into 1 cm^2 into a 500 mL conical flask, washed again with sterile distilled water for 5 times and then blotted on sterilized filter paper. The leaf segments were then plated as done for the endophytic microfungi. A total of 200 leaf segments were plated, 100 each for green leaves and leaf litters. 20 replicate petridishes were used for the green leaves and leaf litters separately.

Frequencies of occurrence of each microfungi on the leaf segments were recorded. A Motic stereo microscope (MZ 168) and an Olympus compound microscope (CX-31) were used for monitoring of fungal reproductive structures and pictures were taken with a hand-held Samsung camera model ES91. Identification of the observed microfungi were made to genus level, and wherever possible, to species level.

Data analysis

The frequencies of occurrence of each microfungal taxa observed was calculated and the frequency of isolation was determined according to (Hata and Futai 1995; Osono 2008) as follows:

$$\text{Freq. of isolation} = \frac{\text{Total no. of leaf segments a fungal taxa was present}}{\text{Total no. of leaf segments observed}} \times 100$$

The diversity indices such as Shannon and Simpson's diversity indices as well as the Bray-Curtis similarity index were calculated using the software Estimates (Colwell 2013).

RESULTS AND DISCUSSION

The total of 74 taxa were identified from 200 leaf segments of *E. zwageri* on WA and MEA, comprising 11 Ascomycetes, 55 anamorphic taxa, 3 Basidiomycetes, and 3 Zygomycetes. Non-sporulating mycelia (*Mycelia sterilia*), both hyaline and melanized were also documented. 67 taxa were identified from green leaves while 20 taxa were from leaf litters (Table 1). The most dominant species observed from both leaf types were *Aphanocladium aranearum, Trichoderma koningii, Nectria* sp., *Chalara pteridina,* Hyphomycetes sp.3, Hyaline *Mycelia sterilia*, *Circinotrichum* sp., *Phoma* sp., *Acremonium macroclavatum, Beltrania rhombica, Chaetopsina* sp. and *Physarum* sp. (Figure 1).

Other species identified includes *Speiropsis pedatospora* (Figure 2.A-C.), *Subulispora longirostrata* (Figure 2.E), *Isthmotricladia* sp., *Fusarium solani, Cylindrocladium* sp., *Dactylaria obtriangularia* and *Monacrosporium* sp (Figure 2G-H). The Shannon and Simpson's diversity indices were higher for the microfungal assemblage on green leaves, 3.85 and 35.93 respectively, than on leaf litters, 2.63 and 11.11 respectively (Tabel 2). This result indicates that leaves of belian support a high diversity of fungi when compared to that recovered from other endangered plant species (Sadaka and Ponge 2003; Shanthi and Vittal 2010; Goveas et al. 2011; Grbić et al. 2015). 41 endophytic fungal taxa were identified from *Coscinium fenestratum* (Goveas et al. 2011)and 49 taxa from *Nepetartanjensis* (Grbić et al. 2015). Also, Sadaka and Ponge (2003) and Shanthi and Vittal (2010) identified 36 and 54 fungal taxa from *Quercus Rotundifolia* and *Pavetta indica* respectively.

Green leaves are usually richer in nutrients than leaf litters (Lodge et al. 2014), thus supporting a more diverse species of microfungi. Green leaves of belian are thick and leathery in texture making it to last longer before decomposing. The high number of fungal taxa from leaves of belian tree altogether shows that this plant species supports the growth of many microfungal species.

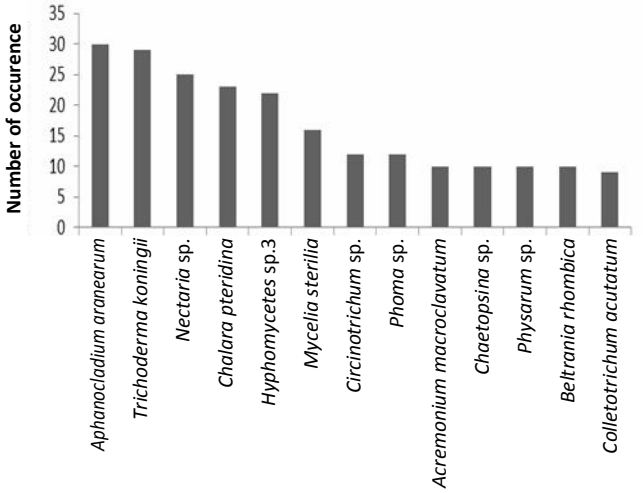

Figure 1. Most dominant microfungal taxa on both green leaves and leaf litters of *Eusideroxylon zwageri* (belian)

Table 1. Percent dominance of microfungi on green leaves and leaf litters of *Eusideroxylon zwageri* (belian)

Microfungal species	Green leaves (%)	Leaf litters (%)
Acladium state of *Botryobasidium consperum*	1.56	3.68
Acremonium macroclavatum Ts. Watan.	3.91	0
Aphanocladium aranearum (Petch) W. Gams	5.86	11.03
Aspergillus nomius Kurtzman, B.W. Horn & Hesselt.	1.17	0
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	1.17	0
Aureobasidium sp.	0.39	0
Basidiomycetes	0.39	0.74
Beltrania rhombica Penz.	1.95	3.68
Bipolaris sp.	0.39	1.47
Bispora sp.	1.17	1.47
Melanized *Mycelia sterilia*	1.17	0
Botryodiplodia sp.	0.39	0
Botrytis sp.	0.39	0
Brachyphoris sp.	1.17	0
Calonectria pyrochroa (Desm.) Sacc.	0.39	0
Calosphaeria cyclospora (Kirschst.) Petr.	0.78	0
Camposporium sp.	0.78	0
Ceriospora polygonacearum (Petr.) Piroz. & Morgan-Jones	0	1.47
Chaetendophragmia sp.	0.39	0
Chaetomium sp.	0.78	0
Chaetopsina sp.	3.91	0
Chaetosphaeria sp.	0.39	0
Chalara pteridina Syd. & P. Syd.	3.13	11.03
Chrysosporium merdarium (Ehrenb.) J.W. Carmich	0.39	0
Circinotrichum sp.	4.69	0
Colletotrichum acutatum J.H. Simmonds	3.52	0
Cryptosporium tami Grove	1.17	0
Cylindrocladium camellia Venkataram. & C.S.V. Rame	0.39	0
Cylindrocladiella parva (P.J. Anderson) Boesew.	0	2.94
Cylindrocladium sp.	1.17	0
Dactylaria obtriangularia Matsush.	0.78	0
Didymella effusa (Niessl) Sacc.	1.56	1.47
Didymella sp.	0.39	0
Diplodia sp.	0.39	0
Drechslera sp.	0.39	0
Epicoccum nipponicum Matsush.	0.39	0
Fusarium merismoides Corda	0.39	0
Fusarium solani (Mart.) Sacc.	1.56	0
Gonytrichum sp.	0.78	0
Harpographium sp.	1.17	0
Hyphomycetes sp.1	0.78	0
Hyphomycetes sp.2	1.56	0
Hyphomycetes sp.3	3.13	10.29
Isthmotricladia sp.	1.56	0
Metacapnodium juniperi (W. Phillips & Plowr.) Speg.	0.39	0
Monacrosporium sp.	0	0.74
Mortierella sp.1	0.39	0
Mortierella sp.2	1.17	0
Calonectria colhounii Peerally	2.73	0
Nectria sp.	1.17	16.18
Neottiosporella sp.	0	2.94
Oidiodendron sp.	0.39	0
Periconia sp.	3.13	0
Pestalotiopsis sp.	0.78	0
Pestalotiopsis versicolor (Speg.) Steyaert	0.78	0
Phaeostalagmus sp.	0.39	0
Phoma sp.	0.78	7.35
Physarum sp.	3.91	0
Piricauda cochinensis (Subram.) M.B. Ellis	1.95	0
Rhinocladiella cristaspora Matsush.	3.52	0
Septonema sp.	0.78	0
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	0.39	0
Speiropsis pedatospora Tubaki	1.17	0
Hyaline *Mycelia sterilia*	4.30	3.68
Subulispora longirostrata Nawawi & Kuthub.	0.39	0
Subulispora procurvata Tubaki	2.34	1.47
Tetrabrunneospora ellisii Dyko	0	1.47
Thielavia sp.	1.56	0
Thozetella sp.	0.78	0
Trichoderma koningii Oudem.	6.25	9.56
Trichosporiella sp.	1.95	0
Vermispora sp.	0	2.94
Veronaea indica (Subram.) M.B. Ellis	0.78	0
Volutella ciliata (Alb. & Schwein.) Fr.	0	4.41

Table 2. Diversity indices and Similarity index of the microfungal communties on green leaves and leaf litters of *Eusideroxylon zwageri* (belian)

Diversity index	Green leaves	Leaf litters
Number of isolates	256	132
Observed species	67	20
Number of Singletons	21	2
Number of Doubletons	12	6
ACE species estimate ± SD	83.09	20.14
Chao 1 species estimate ± SD	83.09	20.14
Shannon Index ± SD	3.85	2.63
Simpson Inv Index ± SD	35.93	11.11

Table 3. Similarity index of microfungi on green leaves and leaf litters of *Eusideroxylon zwageri* (belian)

Samples	Index numbers
Species observed on green leaves	67
Species observed on leaf litters	20
Shared Species Observed	14
Sorensen Classic	0.322
Morisita-Horn	0.442
Bray-Curtis	0.366

Fourteen taxa were commonly identified from both green leaves and leaf litters with a Bray-Curtis similarity index of 0.366 (Table 3). Consequently, there were a total of 53 fungal taxa exclusively identified on green leaves while 7 taxa were from the leaf litters (Figure 3). There were more species exclusively on the green leaves than on the leaf litters which further suggests that the green leaves are more suitable for fungal growth. Furthermore, *Metacapnodium juniperi*, *Phaeostalagmus* sp. and *Monacrosporium* sp. were identified from green leaves and leaf litters respectively, of which these species are not

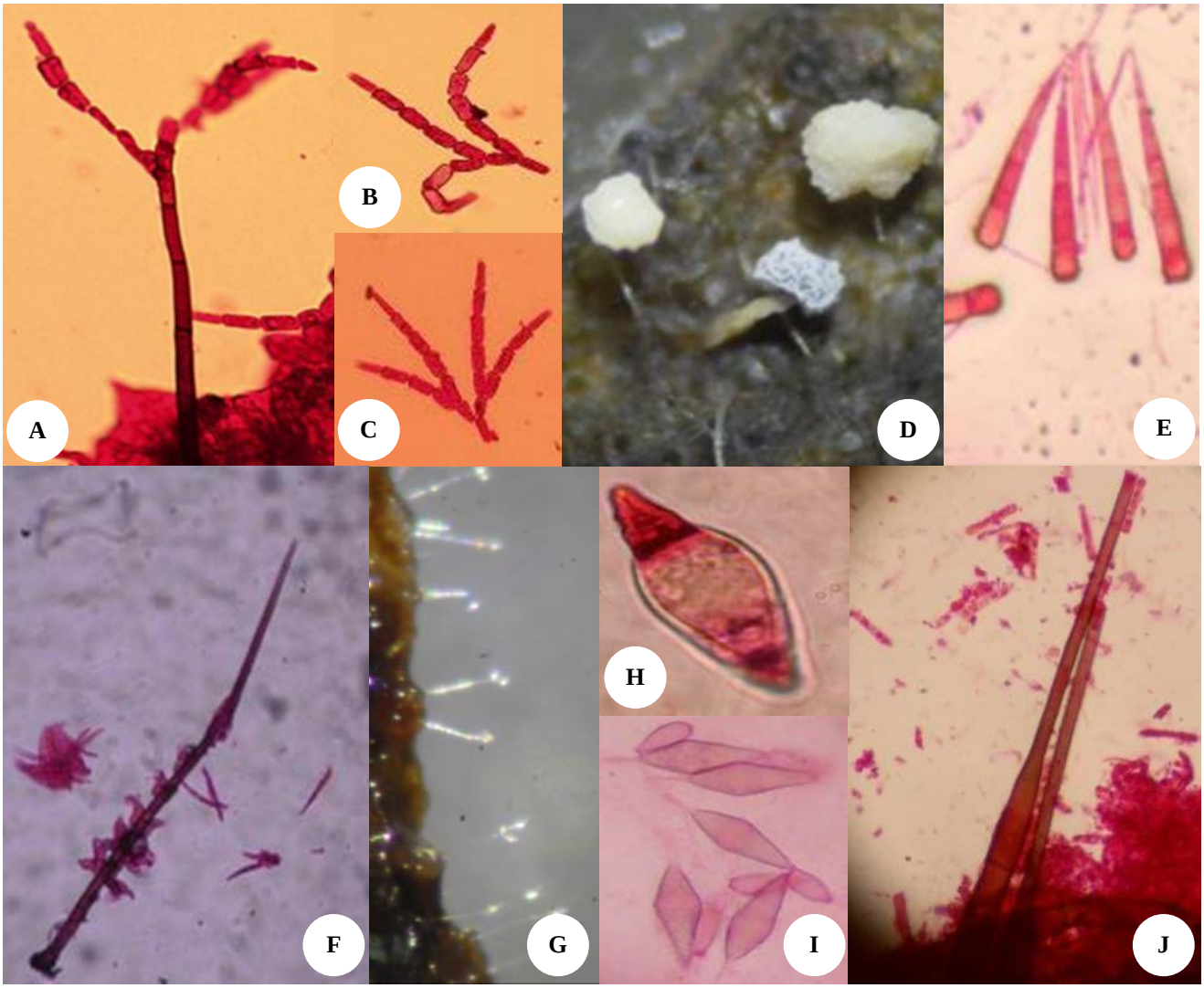

Figure 2. Some microfungal diversity on green leaves and leaf litters of *Eusideroxylon zwageri* (belian). A. *Speiropsis pedatospora*, B - C. Conidia of *S. pedatospora*, D. *Physarum* sp., E. Conidia of *Subulispora longirostrata*, F. *Circinotrichum* sp.,G. Conidiophores of *Monacrosporium* sp., H. Conidia of *Monacrosporium* sp., I. Conidia of *Beltrania* sp., J. *Chalara* sp.

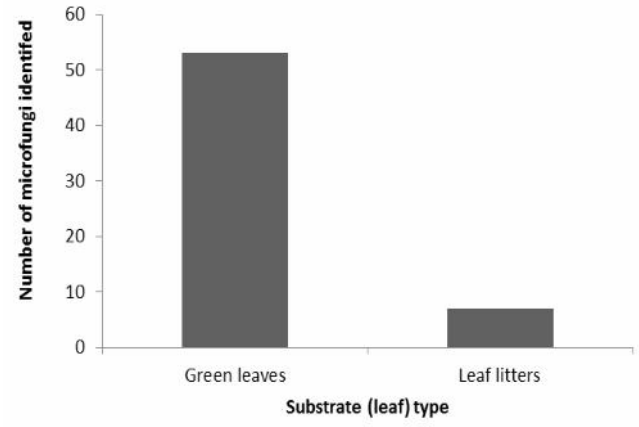

Figure 3. Number of microfungi exclusively observed only on green leaves and leaf litters of *Eusideroxylon zwageri* (belian)

usually frequently isolated from other plant species. Many rare fungal species have been identified on endangered plant species in New Zealand (Buchanan et al. 2002) and also in Sarawak (Hughes 1977). This occurrence signifies the microfungal loss which can be suffered with the extinction of such endangered plant species including belian.

Also, comparison of the fungal communities observed on belian leaves with other plant species in the family Lauraceae showed some similarity in their microfungal communities, in which *Acremonium* sp., *Beltrania* sp., *Chaetopsis* sp., *Chalara* sp., *Dactylaria* sp., *Pestalotiopsis* spp, *Rhinocladiella cristaspora*, *Subulispora* sp., *Thozetella* sp. and *Volutella* sp. have been recorded on leaves of *Cryptocarya mackinnoniana* (Paulus et al. 2006) as saprobic species. *Colletotrichum acutatum* have previously been isolated from *Chlorocardium rodiei* (Cannon and Simmons 2002) and *Cinnamomum bejolghota*

(Suwannarach et al. 2012) as an endophyte. *Fusarium solani*, *Nectria* sp., *Oidiodendron* sp., *Periconia* sp., *Pestalotiopsis* sp., *Phoma* sp. and *Trichoderma* sp. were also recovered from *Cinnamomum bejolghota* (Suwannarach et al. 2012). Other taxa recorded in this study are reported for the first time in the family Lauraceae.

The high demand for wood materials from belian and its slow growth, coupled with its habitat destruction makes it vulnerable to extinction. This study is the first report of microfungal communities on belian leaves in Sarawak. Green leaves supported a higher number of microfungi than the leaf litters. This observation contributes to the understanding of the biology of *E. zwageri* and can be used to strengthen its conservation importance.

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