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Diversity of Arbuscular Mycorrhizal Fungi in Ulu Sat Forest Reserve of Machang District, Kelantan

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ABSTRACT

Bakground: Arbuscular Mycorrhizal (AM) fungi play crucial role in terrestrial symbiosis and one of the important components in soil microbial community. However, little is known about how soil AM fungal community varies in relation to soil properties in Kelantan. **Objective:** Therefore, the present study investigated the types and diversity of AM fungi and proper nutrient composition in soil under trees in native forest of Ulu Sat Forest Reserve of Machang, Kelantan. **Methods:** A total of 60 rhizosphere soil samples were collected from study sites. Then, the rhizospheric soil microfungus was studied using wet-sieving and decanting technique. **Findings/results**: Based on morphological characteristics, 26 AM fungi species were recorded, representing five genera, viz. *Acaulospora* (6 species), *Glomus* (14 species), *Scutellospora* (4 species). *Gigaspora* (1 species), *Sclerocystis* (1 species). *Glomus* was dominant genus in this study site. The AM fungi spore density ranged from 675 to 3020 per 100 g dry soil (average = 1945) and their species richness ranged from 2-9 (average = 5.20). Shannon–Wiener index was calculated to evaluate the AM fungal diversity and the values of species richness (S) and evenness (E) showed a positive correlation with the value of the biodiversity Index. **Conclusion**: The results of this study suggest that the soil physico-chemical properties can have a significant effect on fungal population and diversity. Although tropical rainforests support a high diversity of plants, their associated symbiotic fungi are not as diverse as we had expected, possibly because AM fungi are not specific to their host plant.

INTRODUCTION

The Arbuscular mycorrhizal (AM) fungi are a key, integral component of plant communities in both natural and agricultural ecosystems. The AM fungi form symbiotic relationships or pathogenic associations with plants and animals besides interacting with other microorganisms (Anderson and Cairney, 2004; Zhao *et al*., 2003). They, for example, provide benefit to plants such as improved drought and salinity (Porcel *et al*., 2011; Auge *et al*., 2015), improved growth of crops (Klironomos, 2003), disease resistance (Pozo and Azcon-Aguilar, 2007; Das, 2015), soil quality (Das, 2015), help to control pests and fungal pathogens (Azcon-Aguilar and Barea, 1996). Moreover, plant diversity and productivity in forest ecosystems are influenced significantly by the AM fungal diversity in the soil (Zhao *et al*., 2003; Van der Heijden *et al*., 1998). Also, it has been shown that AM fungi contributed directly to the survival of plant species, affect the fitness of plants in polluted environments and consequently to the equilibrium of ecosystems (Hildebrandt *et al*., 1999). Knowledge of the natural diversity of AM fungi in the root-associated soil in native forest is essential for better management, sustainability and productivity of these tropical ecosystems.

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Ulu Sat Forest Reserve is a tropical rainforest in the region of Kelantan, the country of Malaysia. It has a humid (> 0.65 p/pet) climate and classified as a tropical wet (no dry season), with a tropical moist forest biozone. The land area is not cultivated, where most of the natural vegetation is still intact and covered with closed to open broadleaved evergreen or semi-decidious forest (Forestry Department, 2016). This native forest is considered as a major reserve of biodiversity that supports its ecological functions, besides keeps a valuable secret hidden under its top layers, the so-called fungus garden (Frey-Klett *et al*., 2011). Tropical rainforests are important habitats for AM fungi (Read, 1994), but improper forest management may have significant effect on fungal community and serious implications for both the reestablishment of natural forests and the viability of agro ecosystem (Zhao *et al*., 2003). Therefore, it is important to study the biodiversity of AM fungi as this can improve understanding of tropical forest functioning, plant succession and reforestation in disturbed areas. Thus, the present study investigated the types and diversity of AM fungi in Ulu Sat Forest Reserve of Machang District, Kelantan, Malaysia.

MATERIALS AND METHODS

Soil collection:

Sixty soil samples were collected from different plant rhizospheres to a depth of 5 – 30 cm from Ulu Sat Forest Reserve in Machang District, Kelantan (5.4452º N, 102.2254º E). The samples were collected from 10 random locations and at least 5 m distance between two spot in study site. Soil samples were air dried at room temperature for two weeks and stored in sealed plastic bags at 4ºC before used for the test.

Soil analysis:

Soil samples were transferred to a laboratory from the respective sites. Then, soil sample was air-dried, finely ground, sieved to pass a 2-mm screen. The soil textures were determined by using textural triangle (Anderson and Ingram, 1993). The soil pH was determined using a glass and reference electrode with a pH meter (HI 3220, HANNA Instruments, Inc., 584 Park East Drive, Woonsocket, RI 02895) on a 1:1 suspension (5 g scoop of soil to 5 ml water) (Singh and Ratnasingham, 1977). Soil CEC was determined by ammonium acetate method at pH 7.00 (Chapman, 1965). The determination of soil organic carbon was based on the Walkley-Black chromic acid wet oxidation method (McLeod, 1973). The total bacterial counts were determined by standard spread-plate dilution method described by Seeley and VanDemark (1981) and all the data were expressed as colony forming units (CFU) per gram of dry soil. Meanwhile, chemical analysis for the entire element was determined using Inductively Coupled Plasma (ICP) and elemental analysis.

AM fungi spore isolation and identification:

Wet sieving and decanting method were used to extract AM fungi spores. Twenty grams of soils was suspended in 250 ml of distilled water and stirred with a magnetic stirrer for 10 minutes. Suspension was decanted through a series of 250, 180, 125 and 63 µm sieves. Spores and debris were collected on 125 and 63 µm sieves, washed into a beaker with water, and filtered through filter paper. Clean spores were placed in a 9 cm Petri dish for examination and counted under a dissecting microscope with 40 x magnifications. Each type of isolated spores was mounted in water, lactophenol, PVA and Melzer's reagent, respectively for identification. Criteria for morphological spore characterization were mainly based on spore size and colour, wall structure and hyphal attachment (Morton and Benny, 1990; Schenk and Perez, 1990; Dodd and Rosendahl, 1996; INVAM, 2016).

Statistical analysis:

The different species isolated by means of the soil wet sieving and decanting method were determined at specific levels. The relative frequency of fungal species was calculated as number of fungal spore (or sporocarp) bearing a specific fungus / total number of particles × 100 (Godeas, 1983). Incomplete spores were not scored in the total number of particles. The frequency of appearance of each fungal species was used to calculate the biodiversity index (Shannon-Wiener), H; species richness, S; and evenness, E. Species richness, S, is just the number of different species found in all samples. Species diversity, H, that encompasses both S and E, is quantified according to Magurran (1988).

$$H = \sum_{i=1}^{S} p_i(\log_2 p_i) \quad (1)$$

where *pi* is the probability of finding each species *i* in one sample.

Species evenness, E, that measures the distribution of frequencies for each species in all samples, is given by:

$$E = \frac{H}{\log_2 S} \tag{2}$$

From equation 2 it can be deduced that

$$H = E \log_2 S \tag{3}$$

in which the Shannon–Wiener index appears as the product of the two main components of diversity: evenness and the number of species. Thus, an increased diversity implies not only an augmentation in the number of species but also in the evenness of their distribution (Frontier and Pichod-Viale, 1995).

Pearson correlation was used in order to study the variations in the biodiversity index in relation to evenness (E) and species richness (S).

RESULT AND DISCUSSION

Soil physico-chemical and microbial analyses:

The important physico-chemical properties of the rhizospheric soil at Ulu Sat Forest Reserve are given in Table 1. It is noted that the soil sample was categorized as sandy clay loam soil (24.4% clay, 28.5% silt, and 47.1% sand). The values of organic carbon and CEC are 1.79 and 4.13, respectively. In addition, the soil pH of this soil series is acidic (5.6). Besides, the total bacterial counts in this soil sample were 1.9 x 105 CFU g-1. Meanwhile, chemical analysis for the entire element present in the soil samples were summarized in Table 2.

Earlier work showed that pH (Giovannetti, 2000), organic matter content (Sieverding, 1991; Al-Karaki and Al-Raddad, 1997) and soil N and P availability (Oehl *et al*., 2010; Sheng *et al*., 2013) influence the composition of AM fungi communities and the abundance and occurrence of specific AM fungi species. Result on soil pH (5.6) in this recent study was in line with study conducted by Brundrett *et al*. (1996) who found that the surface soil of natural forest are more acidic than the surface soil of disturbed site. This may be due to the presence of great amount of tannic and humic acids resulting from more active microbial decomposition process in the natural forest. Later on, Premjet and Premjet (2015) found that wood-rotting fungi play a crucial role in litter decomposition, soil humidification, and mineralization of soil organic matter especially in forest ecosystems. Conversely, Taha *et al*. (2016) showed that fungi community in agricultural area significantly affected by soil chemical and physical properties and disturbance.

Table 1: Soil physico-chemicals and microbial analysis.

Parameters	
Soil texture	Sandy clay loam
Clay, %	24.39
Silt, %	28.49
Sand, %	47.12
Organic carbon	1.79
Cation exchange capacity, meq $100g^{-1}$	4.126
pH	5.60
Total bacterial count, CFU g^{-1}	1.9 x 10^5

Abbreviations: meq, milliequivalents; CFU, colony forming units.

Table 2: Soil chemical analysis.

Property	(mg/kg)
Available Mg	280.841
Available K	48.135
Available P	1.047
Available Ca	83.760
Available Cu	2.619
Available Zn	48.258
Available Fe	17732
Available N (%)	0.141

AM fungi composition:

Three-hundred twelve of AM fungal spore (or sporocarp) samples were wet-sieved from the 60 soil samples, from which 26 species of AM fungi were identified (Table 3). The identified species of AM fungi belonged to the genera of *Acaulospora* (6 species), *Glomus* (14 species), *Scutellospora* (4 species), *Gigaspora* (1 species), and *Sclerocystis* (1 species). The results indicated that *Glomus* was the most represented species and

they contributed with 60.9% to the total biodiversity index (H = 1.762). Whereas, *Acaulospora* fungal contribution was 32% (H=0.5647); *Scutellospora,* 6.40% (H=0.1129); *Sclerocytis,* 0.32% (H=0.0056); and *Gigaspora,* 0.32% (H=0.0056). According to Bever *et al*. (1996) and Suresh and Nagarajan, (2010), *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment due to the difference in development. Another studies conducted by Hart and Reader (2002) and Piotrowski *et al*. (2004) found that *Acaulospora* and *Glomus* species require less time to produce spores than *Gigaspora* and *Scutellospora* species.

The species diversity of AM fungi in Ulu Sat Forest Reserve was not as high as we had previously expected, as 26 species of AM fungi were far less than the 60 host plants examined. According to Soka *et al*. (2015) adaptation of species of AM fungi appears to be associated with edaphic or other physical factors and most species have a wide host range. In the present study, many species of AM fungi were found to be common as a study previously conducted by Norhafizah *et al*. (2016). Thus, occurrence of some common species of AM fungi suggests that they exhibit little habitat specificity.

Spore density and species richness of AM fungi:

The distribution of the 26 identified species of AM fungi in the 60 soil samples, the spore density (spores/100g soil) and the species richness per soil sample is given in Table 4. Fungal spore density ranged from 675 to 3020 per 100 g dry soil ($\bar{x}$ = 1945), while their species richness ranged from 2-9 ($\bar{x}$ = 5.20). The spore density was usually positively related to the species richness.

Spore density of five AM fungus genera isolated from soil forest is given in Fig. 1. The dominant genus *Glomus* had the highest spore density of 1856±167.8 spores per 100g soil. According to Bever *et al*. (1996), the variable spore levels are likely due to their differential capacity of each AM fungi species to sporulate. Soil pH in our study was positively correlated with AM fungal and the results did not differ much from previous studies. Soil pH could affect sporulation, spore germination (Wang *et al*. 2004), hyphal growth and root colonization (Medeiros *et al*., 1994) and reproduction and community structure of AM fungi (Sanders *et al*., 1995). According to Norzatushima *et al*. (2015), tropical peat soils are commonly acidic with pH ranging from 3 to 4.3 which would be favorable conditions for fungi growth. The range of pH from 5.5 to 6.5 has been found to favour *Glomus* to sporulate more abundantly in acid soils (Wang *et al*. 1993). However, it is not surprising that most of AM fungi spores owned to the *Glomus* genus because this is the prevailing genus in forest and agricultural soils among the AM species described so far (Jansa *et al*, 2003, Aidar *et al*., 2004).

Table 3: Frequency and species contribution to H index of soil fungal isolated from Ulu Sat Forest Reserve.

No	AM fungi	Occurrence times	Occurrence frequency (%)	Species contribution to H index
	Acaulospora	**100**	**32.04**	**0.5647**
1	*A. spinosa* Walker & Trappe	29	9.29	0.1638
2	*A. denticulata* Sieverding & Toro	29	9.29	0.1638
3	*A. scrobiculata Trappe*	1	0.32	0.0056
4	*Acaulospora* sp.1	13	4.17	0.0734
5	*Acaulospora* sp.2	20	6.41	0.1129
6	*Acaulospora* sp.3	8	2.56	0.0452
	Glomus	**190**	**60.90**	**1.0728**
7	*G. clarum* Nicol. & Schenck	10	3.21	0.0565
8	*G. etunicatum* Becker & Gerd.	3	0.96	0.0169
9	*G. mossae* (Nicol & Gerd.) Walker	19	6.09	0.1073
10	*G. monosporum* Gerd & Trappe	47	15.06	0.2654
11	*G. claroideum* Schenck & Smith	32	10.26	0.1807
12	*G. constrictum* Trappe	20	6.41	0.1129
13	*G. aggregatum* Schenck & Smith	3	0.96	0.0169
14	*Glomus* sp.1	11	3.53	0.0621
15	*Glomus* sp.2	15	4.81	0.0847
16	*Glomus* sp.3	6	1.92	0.0339
17	*Glomus* sp.4	9	2.88	0.0508
18	*Glomus* sp.5	2	0.64	0.0113
19	*Glomus* sp.6	10	3.21	0.0565
20	*Glomus* sp.7	3	0.96	0.0169
	Scutellospora	**20**	**6.40**	**0.1129**
21	*S. heterogama* Walker & Sanders	6	1.92	0.0339
22	*Scutellospora* sp.1	1	0.32	0.0056
23	*Scutellospora* sp.2	4	1.28	0.0226
24	*Scutellospora* sp.3	9	2.88	0.0508
	Sclerocytis	**1**	**0.32**	**0.0056**
25	*Sclerocytis* sp.1	1	0.32	0.0056
	Gigaspora	**1**	**0.32**	**0.0056**
26	*Gigaspora* sp.1	1	0.32	0.0056
	Total AM fungi=26 species	312	100	

Total biodiversity index	1.762

Table 4: AM fungi spore density (SD) and species richness (SR).

Soil Sample	AM fungi	SD	SR	Soil Sample	AM fungi	SD	SR
1	1* 5 12 13 16	1021	5	31	1 9 10 19	1743	4
2	1 2 12 19	1136	4	32	2 5 9 10 11	2372	5
3	1 2 10 12 24	2076	5	33	1 2 5 7 10 11	2735	6
4	4 7 9 14 16	714	5	34	4 5 9 10 19 24	1876	6
5	5 9 19 21	675	4	35	1 10 11 16 24	2078	5
6	2 5 9 10 12	2078	5	36	1 2 5 10 15 16	2356	6
7	2 5 9 14 19	1078	5	37	1 7 10 11 12 14 15	2775	7
8	1 2 9 10	1948	4	38	1 10 11 15	2239	4
9	1 2 9 10 12	2204	5	39	10 12	1249	2
10	2 10 17 21	1545	4	40	10 11 15 17 21	2045	5
11	1 3 10 11	1971	4	41	1 9 10 15 17	2060	5
12	1 9 10 11 12 14 19 24	2557	8	42	7 9 10 11 14 19 20 23	2276	8
13	4 7 11 17 23 24	1189	6	43	1 2 8 9 10 15 20	2325	7
14	5 10 11 12 19 21 24	2346	7	44	2 5 10 11 15 16	2499	6
15	1 7 15	861	3	45	2 4 5 11 12 17	1766	6
16	1 2 6 10 11 19	2645	6	46	4 10 15 18	1468	4
17	1 5 7 10 11 12 15	2941	7	47	2 5 10 11 13	2193	5
18	1 2 4 6 12 24	1483	6	48	9 10 11	1752	3
19	1 2 5 6 8 9 10 17	2644	8	49	7 9 11 20 24	1062	5
20	1 2 9 10 11 12 17 21 23	3020	9	50	1 2 10 21	1790	4
21	2 10 12 17	1750	4	51	4 6 11	950	3
22	6 10 11 15 17 23	2269	6	52	10 11 15 19	1966	4
23	2 5 10 12 18	1877	5	53	2 4 6 10 11	2282	5
24	1 2 4 5 10 11 12	3004	7	54	1 10 11 14	2065	4
25	7 11 12 15	1260	4	55	4 7 10 11 12	2142	5
26	7 10 14	1273	3	56	9 10 12 15 16	1794	5
27	1 2 4 5 10 11 12	3004	7	57	5 10 11 13 24 25	1936	6
28	1 2 4 10 14	2032	5	58	1 4 10 11 14	2243	5
29	1 2 5 6 10	2242	5	59	10 11 14 15 26	1948	5
30	1 2 5 6 11 14	1914	6	60	1 2 9 10 22	1949	5
Total: soil samples = 60; Average spore density = 1945 ± 582; Species richness = 5.2 ± 1.4							

*Numbers in this column refers to the codes of AM fungi species in Table 3.

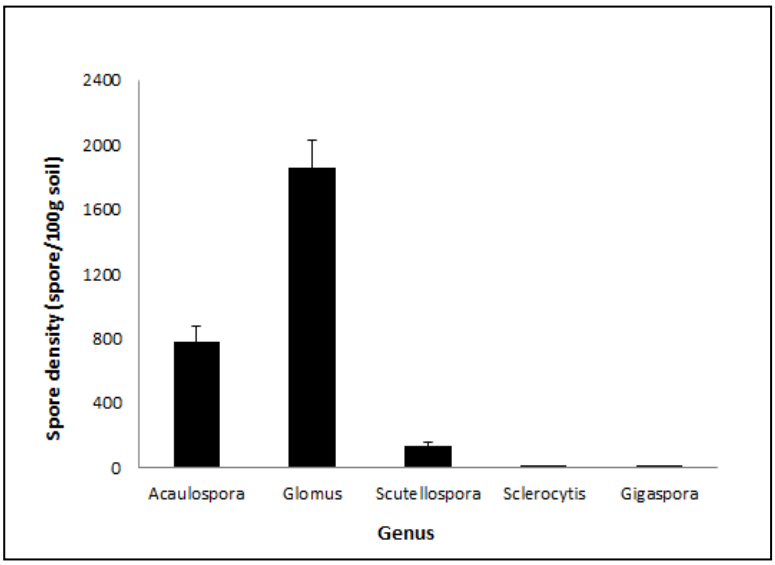

Fig. 1: Spore density of five AM fungi genera isolated from Ulu Sat Forest Reserve. Values are means of 5 replicates. Error bars = Standard error.

AM fungi species distribution:

Table 5 shows the biodiversity index (H), the richness (S) and the evenness (E) for fungal isolated from Ulu Sat Forest Reserve. The values of species richness (S) and evenness (E) showed a positive correlation with the value of the biodiversity Index (H) ($r = 0.58$, $P<0.001$ and 0.92, $P<0.001$) (Fig. 2). A study conducted by Moreira *et al*. (2007) showed that the higher value of H in the native forest reflects greater species diversity. These findings can probably be explained by better soil chemical properties of this area with regard to organic

matter, pH, Ca and Mg (Kernaghan, 2005; Wilson *et al*., 2009). According to Brodie *et al*. (2003) and Pfenning (2006), a high diversity of plants could promote greater microbial species richness due to the greater number of niches in the rhizosphere or specific interactions between plants and microorganisms. Besides, species richness was the most important component to explain the ensuing values of diversity. This fact agrees with Persiani *et al*. (1998) who found that the biodiversity correlates better with evenness, because they had a variable number of species but evenness tended to be quite high in the majority of cases.

Table 5: Biodiversity index, evenness and species richness values for AM fungi species found at Ulu Sat Forest Reserve.

Index	Value
$H = \sum_{i=1}^{S} p_i(\log_2 p_i)$	1.762
$E = \frac{H}{\log_2 S}$	0.540
S =	26

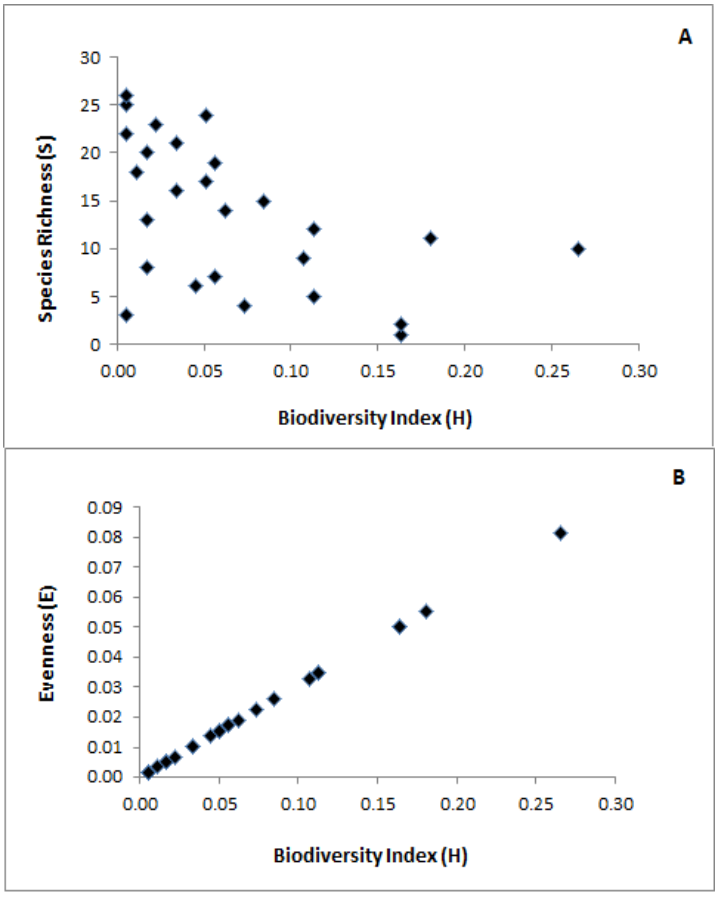

Fig. 2: Variation in the Shannon-Wheaver diversity index in relation to (A) Species richness, S, and (B) evenness, E for soil fungal communities from Ulu Sat Forest Reserve.

Conclusion:

The AM fungi are the most-important symbionts, being a key, integral component of plant communities in both natural and agricultural ecosystems. In this study, 26 species of AM fungi belonging to five genera i.e. *Acaulospora* (6 species), *Glomus* (14 species), *Scutellospora* (4 species), *Gigaspora* (1 species) and *Sclerocystis* (1 species) were collected and identified. The genus *Glomus* was the most common AM fungi in the soils of study areas. We conclude that Ulu Sat Forest Reserve contained a high AM fungal diversity where the complex below ground structure including soil properties of tropical rainforests is major factors that affect the spore density. This study indirectly can catalogue the AM fungi species so that we can use in future for restoration and regeneration of degraded forests and maintenance of sustainable forestry. Hence, by providing assistance to

agricultural practices, the results of the present work may extend the existing knowledge to further study the interaction effects of AM fungi and soil applied herbicides on plant growth. Nowadays, interaction between AM fungi and herbicide application and their effects on plant growth are issues around which there is little information. This new study will help us to understand the capability of AM fungi in enhance the crop growth under stress condition.

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