Journal of Academia Vol. 10, Issue 1 (2022) 11 – 21

DIVERSITY OF MACROFUNGI IN UITM FOREST RESERVE, KUALA PILAH, NEGERI SEMBILAN

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Abstract

Macrofungi have perhaps the longest history of diversity studies, yet they are understudied over most of the world. They perform some of the significant parts in life and also have advantages for biotechnology, nevertheless only 7% of the total estimated fungal species are reported worldwide. The diversity of fungi is greatly influenced by biotic and abiotic factors. Different families and species have different nutritional modes and are associated with substrates that offer a preferable condition to grow. In UiTM Forest Reserve, Kuala Pilah, Negeri Sembilan, the diversity of macrofungi is poorly documented and unexplored taxonomic diversity resulted in a lack of awareness of their importance in the ecosystem. Thus, this study aimed to identify families, genera and to determine the diversity of macrofungi in the UiTM Forest Reserve. This study may gain knowledge about the diversity of macrofungi that has been recorded in this area and contribute towards conservation together with preservation efforts on ecosystem health and forest resources as a need for sustainable development. The sampling activity was conducted four times in April 2021 and the samples were photographed before being recorded based on morphological features. The specimens were placed into ziplock bags separately then were brought to the laboratory for the identification process. In total, five orders and ten families were identified from 160 samples collected. The highest number of fruiting bodies contributed by Agaricales by 81 from six families and 15 species, followed by Polyporales, Auriculariales, Hymenochaetales and Hypocreales with 57, 17, 4 and 1 fruiting bodies respectively. The statistical analysis shown the diversity (H) = 2.54, evenness (E) = 0.58 and richness (R) = 4.14 of macrofungi diversity respectively. UiTM Forest Reserve thus has high macrofungi diversity. However, further thorough studies are required to document more on macrofungi diversity within this area.

Keywords: diversity; macrofungi; UiTM Forest Reserve

Article History:- Received: 28 August 2021; Accepted: 2 December 2021; Published: 30 April 2022

Introduction

Mushrooms have always been misunderstood as a type of plant, yet they are grouped under Kingdom Fungi. Macrofungi are also called macromycetes due to visible spore-bearing organ which is fruiting bodies, while plants do not have this structure (Bakray *et al.*, 2020). According to Hawksworth and Lücking (2017), about 2.2 to 3.8 million macrofungi species have been recorded globally. Fungi are grouped into two major phyla; Ascomycota and Basidiomycota (Mohammad *et al.*, 2019). Macrofungi can be found in most types of habitats, depending on the composition of tree species and other substrates (Parveen *et al.*, 2017). The authors also reported that water supply could be the factor of production of basidiomes based on their occurrence and frequency. In addition, knowledge on macrofungi is poorly documented at UiTM Forest Reserve. Macrofungi often be neglected in biodiversity compared to plants and this might be caused by unexplored taxonomic and insufficient information on diversity, distribution and awareness on their importance in ecosystem. Therefore, this study attempted to document the diversity and distribution of macrofungi in UiTM Forest Reserve, Kuala Pilah, Negeri Sembilan. This research will be a significant endeavour to community to understand better fungal community structure and the importance in the forest in decomposing, recycling all the nutrients and mutualistic interaction with other organism in order to balance the ecosystems. This research will also be beneficial to contribute towards conservation and preservation efforts on ecosystem health and forest resources as a need for sustainable development. From the literature review that has been done, limited study is conducted on the diversity of macrofungi in Kuala Pilah, Negeri Sembilan.

Methods

Study Area

UiTM Forest Reserve in Kuala Pilah, Negeri Sembilan was chose as sampling site and the geocoordinate was at 2°47’28.0”N 102°13’11.7”E. Characteristics of tropical rainforest in Malaysia including having regular rainfall in between 2000 to 2500 mm per year and temperature of 29 ºC to 38 ºC (Hazir *et al*., 2020). The sampling method was done according to Shujiang *et al.* (2012), using the square quadrat of 20m x 20 m. The site was identified for sampling collections with damp soil and abundant of leaf litters (Kaik and Mohammad, 2019). This study was conducted four times in April 2021.

Collection, preservation and identification methods

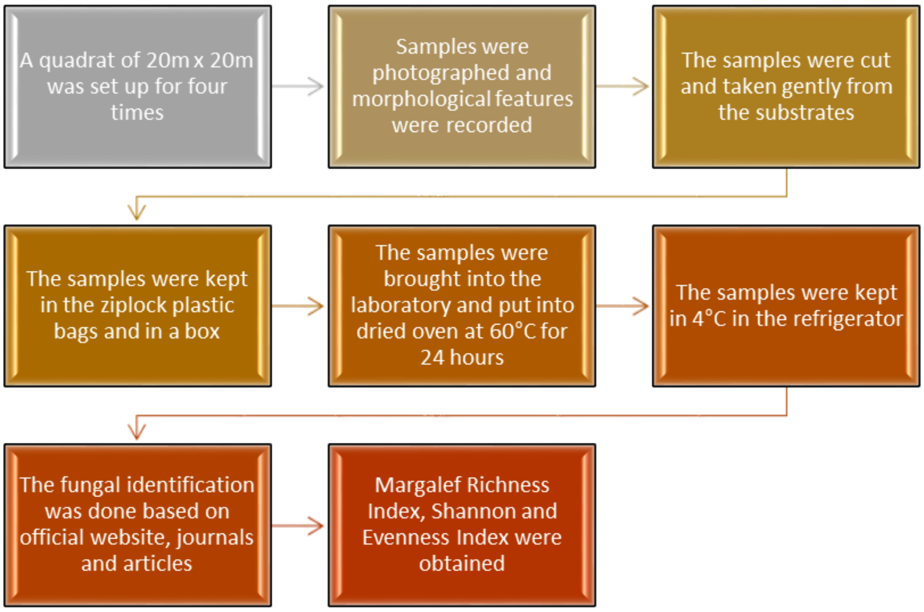

Figure 1. Experimental flowchart

Figure 1 shows an overview of the experimental flowchart in this study. A sampling of macrofungi was carried out in a quadrat using a measuring tape 20m x 20m in UiTM Forest Reserve for four times. All macrofungi samples were photographed before being recorded based on morphological features including colour, number of fruiting bodies and substrates because some of the characteristics might change with drying (Mohammad *et al.*, 2019). Samples were observed and collected on fallen rotting branches, twigs, dead trees and leaves litters (Kaik and Mohammad, 2019). Soft macrofungi were cut using a knife, while the tree-dwelling samples were carefully cut off to prevent spore surface disturbance below (Parveen *et al.*, 2017; Priyamvada *et al*., 2017). The specimens were placed into ziplock bags separately and kept in a box since drying and spore contamination between each sample must be prevented. The specimens then were brought to the laboratory and dried in an oven at 60°C for 24 hours or more, until the specimens were fully dried. As moderate temperatures were used, thus the specimens were not killed but dormant and they would be freeze and kept in the refrigerator at 4°C for next identification (Kinge *et al.*, 2017). Each of the specimens was rinsed with distilled water and pat dried by using tissues. Then, the specimen was put on the white tile and macroscopic features of the fruiting body such as colour and shape of pileus, lamellae and presence of stipe were observed and recorded for species identification (Mohammad *et al.*, 2019). After that, the hymenium of specimens was observed under a compound light microscope. A brief analysis of the fungal identification was done based on macroscopic features and substrates through official websites such as First Nature, Mushroom Database, journals and articles such as Kin and See (2008) in Proceeding of National Biodiversity Seminar 2008, Salim *et al.* (2011) in Terengganu Natural Ecosystems Series, Checklist of Fungi in Malaysia by Lee *et al.* (2012), Journal of Tropical Biology and Conservation by Foo *et al.* (2018), Philippine Journal of Systematic Biology by Paguirigan *et al.* (2020) and some others.

Statistical analysis

All three values of Margalef Richness, Shannon Weiner's Diversity and Evenness were calculated by using Past4.03. Margalef Richness Index (R) was used in this study to calculate the species richness. It was a good indicator of diversity and a relevant parameter in estimating the pattern series of data (Iglesias-Rios and Mazzoni, 2014).

$$d = \frac{S - 1}{\ln N}$$

Shannon Weiner's Diversity Index (H) was also used in this research (Enow *et al.*, 2013; Shujiang *et al.*, 2012). The pi is the proportion (Ni/N) of individuals of one particular species found (Ni) is divided by the total number of individuals found (N). A data of ln pi for each species found is calculated and multiplied with pi to obtain data of pi ln pi. The sum of pi ln pi was added and Shannon Index was obtained.

$$\sum_{i=1}^{s} p_i \ln p_i$$

Evenness Index (E) was used which H value was derived from (H) and S is the total number of species.

$$\frac{H}{\ln S}$$

Result and Discussion

Collections

Table 1. Collections of macrofungi in UiTM Forest Reserve

Scientific name	No of fruiting body	Substrate	Color
Agaricus sp.	2	Soil	Brownish white
Amanita sp. 1	1	Soil	Brownish
Amanita sp. 2	1	Soil	Pale yellowish
Auricularia sp.	17	Log	Brownish black
Beauveria sp.	1	Living tree	White
Bovista sp.	4	Soil	White
Calvatia sp. 1	4	Soil	Brownish white
Calvatia sp. 2	15	Soil	Brownish white
Coltricia sp.	4	Soil	Orange
Gerronema sp.	1	Decayed branches	Yellowish
Leucocoprinus sp.	2	Soil	Yellowish white
Lentinus sp.	4	Decayed branches	Pale brownish
Lycoperdon sp.	12	Soil	White
Marasmiellus sp.	11	Living tree	Yellowish white
Microporus sp. 2	9	Log	Orange
Microporus sp. 1	42	Living tree	Brownish orange
Mycena sp.	2	Soil	Brownish
Termitomyces sp.	2	Soil	Brownish white
Trametes sp.	2	Log	Brownish white
Parasola sp.	10	Soil	White
Psathyrella sp.	2	Soil	Pale brownish
Xeromphalina sp.	12	Log	Brownish orange
Total 22	160		

Table 2. Order and Family of macrofungi found in UiTM Forest Reserve

Order	Family	Scientific name	No of fruiting body
Agaricales	Agaricaceae	*Agaricus* sp.	2
		Bovista sp.s	4
		Calvatia sp. 1	4
		Calvatia sp. 2	15
		Leucocoprinus sp.	2
		Lycoperdon sp.	12
	Amanitaceae	*Amanita* sp. 1	1
		Amanita sp. 2	1
	Lyophyllaceae	*Termitomyces* sp.	2
	Marasmiaceae	*Gerronema* sp.	1
		Marasmiellus sp.	11
	Mycenaceae	*Mycena* sp.	2
		Xeromphalina sp.	12
	Psathyrellaceae	*Parasola* sp.	10
		Psathyrella sp.	2
Auriculariales	Auriculariaceae	*Auricularia* sp.	17
Polyporales	Polyporaceae	*Lentinus* sp.	4
		Microporus sp. 1	42
		Microporus sp. 2	9
		Trametes sp.	2
Hymenochaetales	Hymenochaetaceae	*Coltricia* sp.	4
Hypocreales	Cordycipitaceae	*Beauveria* sp.	1

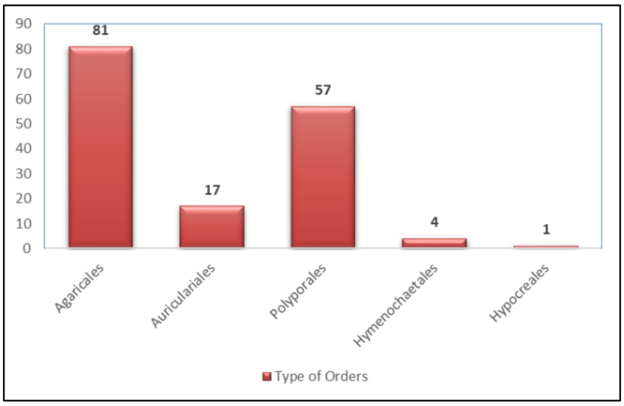

Figure 2. Type of Orders and number of fruiting bodies

The collection of samples in the UiTM Forest Reserve were recorded in Table 1 including the scientific name, number of fruiting bodies, substrates and colours Five orders and ten families were identified from 160 samples collected. Orders of Agaricales, Auriculariales, Hymenochaetales, Hypocreales and Polyporales together with families of Agaricaceae, Auriculariaceae, Polyporaceae, Hymenochaetaceae and Cordycipitaceae as shown in Table 2 were observed in UiTM Forest Reserve. Only one order belonged to Ascomycota, while the rest were from Basidiomycota. Ascomycota represents the least number since only a fruiting body of *Beauveria* sp. was found in this study. Even though Agaricales made up to 81 fruiting bodies from 15 species, Polyporales recorded to have 57 samples and species of *Microporus* sp. 1 contributed up to 26%, which is a quarter out of the total samples. Four species of *Amanita* sp. 1, *Amanita* sp. 2, *Gerronema* sp. and *Beauveria* sp. made up the least number of fruiting body by only a sample. Agaricales, contributed the highest number of samples with 81, followed by Polyporales, Auriculariales and Hymenochaetales, with 57, 17 and 4 samples respectively as in Figure 2. This is shown that UiTM Forest Reserve had more diversity in Agaricales compared to other orders.

Bakray *et al.* (2020) reported that 6 families and 13 genera were observed from 107 samples and only 90 samples were identified in Gunung Korbu, Perak. Polyporaceae with 63 fruiting bodies, the highest from other families, consisted of *Trametes* and *Microporus*, which also were recorded in this study. The authors mentioned, plenty of Polyporaceae in the study might be from the availability and preferable substrates for the growth of macrofungi. Thirty-one fruiting bodies of *Microporus* sp. were collected in Gunung Korbu, the highest compared to other species, the same outcome in UiTM Forest Reserve. Both *Microporus* sp. 1 and *Microporus* sp. 2 sums up to had the greatest number of fruiting bodies by 51 as in Table 2. However, five genera had been identified in Gunung Korbu, while only three genera in UiTM Forest Reserve had only common in *Trametes* (Figure 3) and *Microporus* (Figure 4).

Figure 3. *Trametes* sp.

Figure 4. *Microporous* sp

Other than that, the same genera of *Agaricus, Amanita, Auricularia, Gerronema, Lentinus, Marasmiellus, Microporus, Mycena, Termitomyces* and *Trametes* were also found in Pulau Sibu. In this study, the family Agaricaceae has shown the highest number of genera collected with five including *Agaricus, Bovista, Calvatia, Leucocoprinus* and *Lycoperdon* but Polyporaceae was the highest number of genera collected in Pulau Sibu. Polyporaceae of genera *Lentinus, Trametes* and *Microporus* were found in both Pulau Aur and Pulau Sibu but with higher number of genera of 9 and 11 respectively compared to only three genera in UiTM Forest Reserve (Mohammad *et al.*, 2019). The authors also mentioned that most of the family of Agaricaceae, Marasmiaceae and Mycenaceae were the saprophytic fungi while Amanitaceae was ectomycorrhizal fungi which these four families were found in UiTM Forest Reserve.

Marasmiaceae, Lyophyllaceae and Psathyrellaceae were recorded in both studies while families of Cortinariaceae, Pluteaceae and Schizophyllaceae were not observed in UiTM Forest Reserve. Even so, Agaricales had shown as the highest number of families in study by Putra (2020) and this was formerly also found in the UiTM Forest Reserve. The author added, that Agaricales is known as worldwide macrofungi since they were much easier to find in a wide range of habitats. They also were crucial in biodegradation and biodeterioration.

Samples of Polyporales in this study were much lower than at recreational forest in Negeri Sembilan which had recorded 19 species arising from four families (Bolhassan *et al.*, 2012). The only family in UiTM Forest Reserve was Polyporaceae, while additional Fomitopsidaceae, Ganodermataceae and Meripilaceae were found in Negeri Sembilan Recreational Forest. Fourteen species from Polyporaceae found in Negeri Sembilan Recreational Forest including *Lenzites elegans*, *Earliella scabrosa* and *Pycnoporus sanguineus* but unobserved in UiTM Forest Reserve. This might be explained as six years were used in the Negeri Sembilan Recreational Forest study instead of just one month in UiTM Forest Reserve. The authors mentioned as occurrence and species diversity were directly proportional to the number of visits over a longer period. Besides that, in their study, *Lentinus* spp. were commonly found in both Langkawi and Selangor while *Microporus* sp. was found in both Pahang and Selangor. The authors added, only 29 species of macrofungi were observed in Negeri Sembilan and Selangor had the highest collection of macrofungi which could be explained that collections were done regularly during the study period. The authors also suggested, other factors such as rainfall, amount of preferable substrate and wet forest with constant high humidity might cause a low collection of Polyporaceae.

Only two species of ascomycetes which were *Cookeina* and *Xylaria* found in Pulau Aur, Johor, a species of *Galiella* in Pulau Redang, Terengganu and a basidiocarp *Xylaria polymorpha* was found in Pulau Sibu proved that ascomycetes are less dominant compared to basidiomycetes (Mohammad *et al.*, 2019). The least number of Ascomycetes was also recorded in Bicol University Kalikasan Forest Park, Philippines which were *Aleuria* sp., *Philipsia* sp., and *Xylaria* sp. (Guerrero *et al.*, 2020). Furthermore, a study by Paguirigan *et al.* (2020) also recorded the least number of Ascomycetes with only seven species in Ascomycetes while 32 species belonged to Basidiomycetes at Consocep Mountain Resort and Mount Isarog National Park which made up to 39 species including unidentified species. The dominance of Basidiomycetes might be caused by preferable substrates, right temperature, humidity and soil that provided the best growth development of macrofungi. The authors also added the high number of species might be the presence of a waterfall, river, high diversity of trees and accumulation of dead plant materials. From the previous studies, *Xylaria* sp. was common in Pulau Aur, Pulau Sibu, Bicol University Kalikasan Forest Park, Consocep Mountain Resort and Mount Isarog National Park but undiscovered in UiTM Forest Reserve. Even so, a study by Noormi *et al.* (2018) found dominancy in ascomycetes including orange sea lichen, button jelly lichen and tree-dwelling lichen with 6 in total and 5 of basidiomycetes. The high occurrence of lichens might arise from anthropogenic activities at the study area of UiTM Kuala Pilah.

Macrofungi species richness and diversity

Table 3. Diversity index

Title	Value
Number of species	22
Number of fruiting bodies	160
Margalef Richness Index (R)	4.14
Shannon Weiner's Index (H)	2.54
Evenness index (E)	0.58

R value of 4.14 was obtained as shown in Table 3 which was slightly lower than a study in Garbhanga Reserve Forest in India that obtained a value of 5.91 during rainy season (Devi, 2017). The main reason was only 22 species were recorded in UiTM Forest Reserve compared to 37 species in Garbhanga Reserve Forest. The same families found in both forest reserves were Agaricaceae, Auriculariaceae, Hymenochaetaceae, Lyophyllaceae, Marasmiaceae, Polyporaceae and Psathyrellacea. However, the R values dropped to 1.13 and 0.74 respectively during summer and winter seasons in Garbhanga Reserve Forest which lower than in UiTM Forest Reserve. This is because of higher number of fruiting bodies and species richness could be found in area that had a high rainfall (Jarvis *et al.*, 2017). According to Priyamvada *et al.* (2017), greater number of species can be found at areas that have suitable temperature, season and also the amount of rainfall at optimum. Besides that, a study by Li *et al.* (2018) mentioned that higher temperature and rainfall in the tropical regions had contributed to a prolonged interval of macrofungal fruiting. This proved that rainy season obtained higher R value compared to summer and winter seasons. High temperature and rainfall in tropical areas might bring to prior energy accumulation of macrofungal development. The authors also added that macrofungal fructification also effected by precipitation. Thus, this shown that rainfall, temperature and humidity could affect the distribution of macrofungi in certain areas. However, lack of study regarding R values of macroufungi in Malaysia. Furthermore. a study by Patel and Trivedi (2017) also shown higher R value of 8.74 in Waghai, India since it collected 31 species which was more than species in UiTM Forest Reserve. This shown R value increased with the number of species collected. Higher R values of 13.42, 8.74 and 4.56 shown at Kogi East, Kogi West and Kogi West can be explained as bigger sampling site covered and longer sampling period of three years, instead of only a month in UiTM Forest (Apollos *et al*., 2017).

This study gained H value of 2.54 in Table 2 while Bakray *et al.* (2020) gained a value of 2.20 on Trail B at Gunung Korbu. H value that ranges in between 1.5 and 3.5 displayed a high diversity which both UiTM Forest Reserve and Gunung Korbu were obtained. Thus, UiTM Forest Reserve had higher diversity as there were 22 species found compared to only 13 species including three unidentified species in Gunung Korbu. Study by Kaik and Mohammad (2019) gained (H) values of 2.67 and 3.14 for Trail A and Trail B respectively at Pulau Bidong which both of the values indicate high diversity of macrofungi since index value of 4 showed the highest diversity and decreases as the value decreased. This shown the diversity in UiTM Forest Reserve was slightly lower than in Pulau Bidong.

As for E value in Table 3, macrofungi in this study did not prove either strong or weak uniformed to each species since the value was 0.58, similar trend as 0.54 for Trail A and 0.59 for Trail B at Pulau Bidong (Kaik and Mohammad, 2019) indicated that not all species were distributed evenly. Value of one expressed high evenness and decreases as the value reached to zero. However, study by Bakray *et al.* (2020) mentioned Gunung Korbu had nearly strong uniformed species distribution as the values expressed were 0.88 and 0.82 from Trail A and Trail B respectively. This proved that E value in UiTM Forest Reserve had lower evenness for each species compared to E values at Pulau Bidong.

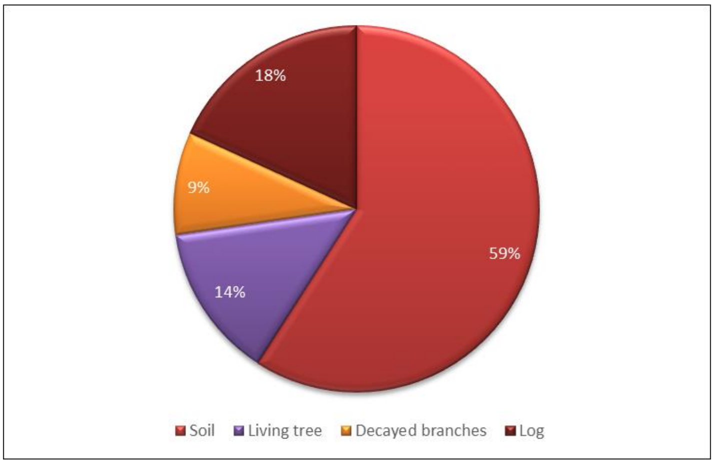

Figure 5. Type of substrates being collected

Substrate decomposition is a crucial process for macrofungi acquiring energy and nutrients (Ye *et al*., 2019). There were four types of substrates observed based on Figure 5 including log, soil, decayed branches and living tree. 13 out of 22 species were found on soil which resulted it as the most suitable substrate within the samples with 59%; more than a half from total samples and thrice from the substrates on log which was 18%. A study in Pulau Sibu was also recorded that *Agaricus*, *Amanita* and *Termitomyces* preferred soil as substrates while *Auricularia* was found on dead wood same findings in this study. In meanwhile, living tree had slightly less occurrence of samples than on logs by 14% and decayed branches had percentage of only 9% which was the least occurrence of samples in this study. *Calvatia* sp. 2 contributed the highest fruiting bodies that found on soil by 15 while the most popular samples preferred log as their substrate was *Auricularia* sp. with 17 basidiocarps. Furthermore, the most suitable species lived on living tree conquered by *Microporus* sp. 1 by 42 and the highest number of basidiocarps collected among all of the samples. Both of *Gerronema* sp and *Lentinus* sp. chose decayed branches to grow; with one and four basidiocarps respectively. However, study by Kaik and Mohammad (2019) stated only 10 species were found on soil in Pulau Bidong compared to 12 species in UiTM Forest Reserve. 30 from 65 species were found on decayed branches which was the highest among to other substrates in Pulau Bidong. The diversity of forest community structures such as nutrient compositions and substrate sizes that accessible for macrofungi growth could be a reason of high occurrence of specimens on the decayed branches. Even so, more type of substrates was recorded in Pulau Bidong by six including termite mounds and decayed leaves which were not reported in UiTM Forest Reserve.

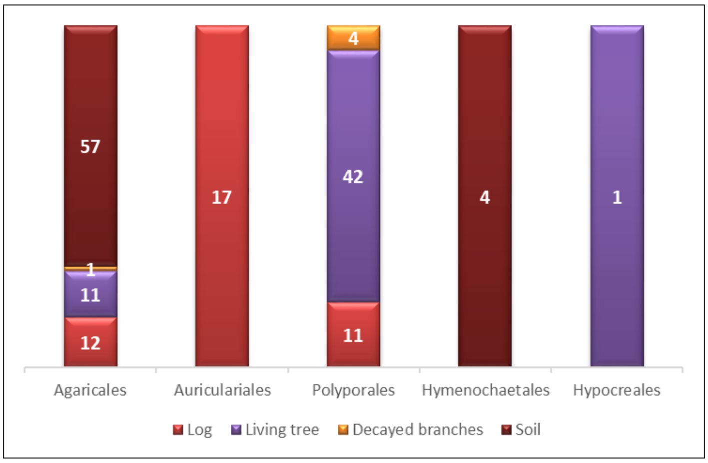

Figure 6. Type of substrates of orders

Moving on to Figure 6, clearly shown Agaricales had all four preferable substrates; 11 and 12 at living trees and logs as the most samples found was on soil with 57 basidiocarps. Only a basidiocarp was found on decayed branches which was *Gerronema* sp. The Auriculariales, Hymenochaetales and Hypocreales had only one dominant substrates; Auriculariales on logs by 17 samples while Hymenochaetales and Hypocreales had the same substrate on living trees with 42 and 1 samples respectively. Polyporales was more dominant on living tree by 42 samples compared to 11 and 4 on logs and decayed branches respectively. This study proved the soil became the most preferable substrate for macrofungi to grow and reproduce in UiTM Forest Reserve. Moreover, study by Seelan *et al*. (2014) found a contradict finding where the highest occurrence of samples on substrates was dead trees with 42.6% but only 5% for soil. This might due to different slightly in temperature, humidity and soil pH which could lead to low sporocarps production. Insufficient time and area covered for sampling also might contribute to different results from one another. Study by Mohammad *et al*. (2019) also found more than 80% of the samples were from dead wood and leaf litters. Besides that, the samples such as agarics were collected from the soil but not dominant as wood-inhabiting fungi.

Conclusion

Roles in decomposition, nutrient cycling and mutualistic relationships provided by macrofungi are essential for ecosystems (Mohammad *et al.,* 2019). To conclude, UiTM Forest Reserve owned a high diversity on macrofungi especially in Basidiomycota same as other studies in Terengganu and Johor. Even though macrofungi in this area is not threatened, but then they should be conserved and preserved so that these macrofungi could be protected for future medicines or development. With this study, in hopes that an awareness to society regarding macrofungal diversity is delivered successfully. Five orders and ten families were identified and the most abundant species contributed by Order Agaricales. The statistical analysis shows the diversity (H) = 2.54, evenness (E) = 0.58 and richness (R) = 4.14 of macrofungi diversity respectively. UiTM Forest Reserve has quite high macrofungi diversity. However, the macrofungal diversity within this area is not fully understood. Hence, it is suggested that more studies regarding diversity of macrofungi in UiTM Forest Reserve would be done in order to have advanced collections and results in this area for the time being. The findings of this study could be used as baseline information for assessing the biodiversity (Noormi *et al.*, 2018).

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