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Mushrooms Assumed as Ectomycorrhizal Fungi on South Kalimantan Serpentine Soil

Hermawan R, Imaningsih W, Badruzsaufari

Biology Study Program, Mathematics and Natural Science Faculty, Lambung Mangkurat University, Banjarbaru, South Kalimantan

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Abstract

Serpentine soil contains highly heavy metals, such as manganese, chromium, cobalt, and nickel, which could be an inappropriate growth media of most plants. Some plants that found able to grow optimally on South Kalimantan serpentine soil have been known to do association with ectomycorrhizal fungi. This research aimed to obtain and characterize mushrooms assumed as ectomycorrhizal fungi indigenous South Kalimantan serpentine soil. This study used field exploration of fungal fruiting bodies and identified the genus based on morphological characters of fruiting bodies such as shape, size, and ornamentation, which are unique for the genus identification, then compared the characteristics on mushroomexpert.com. The mushrooms were also confirmed of genera assumed as ectomycorrhizal fungi based on mycorrhizas.info. Seven fruiting bodies were obtained and classified as *Cantharellus* (Ct), *Chlorophyllum* (Ch1 and Ch2), *Lycoperdon* (Ly), *Ramaria* (Rm1 and Rm2), and *Thelephora* (Tp). The results showed that all of those fruiting bodies belong to *Basidiomycetes*. There were 4 genera of *Cantharellus*, *Lycoperdon*, *Ramaria,* and *Thelephora,* assumed *as* ectomycorrhizal fungi. But *Chlorophyllum* genus was never reported as ectomycorrhizal fungus.

Keywords – *Basidiomycetes* – indigenous – mushroom

Introduction

Serpentine soil is derived from the weathering of ultramafic rock that contains a high concentration of heavy metals such as Cr, Mn, Co, Ni, Mg, and Fe, but the low concentration of essential elements, for examples, Ca, N, and P (Ross, 1994; Kayama et al., 2005). As a result, in addition to its effects on metal toxicity, it causes plant nutrition such as calcium (Ca) to become very limited. South Kalimantan serpentine soil scatters along the ridge of Meratus Mountain (Gorsel, 2013). Some spots are serpentine soil of Mandiangin and Awang Bangkal of South Kalimantan, which contain Cr, Ni, and Mn in concentration up to 1,500, 7,000, and 4,000 $mg.kg^{-1}$ respectively (Saidy & Badruzsaufari, 2009).

The harsh characters of serpentine soil give rise to restrict the growth of most plants. However, some plants, especially endemic plants, found grow optimally on serpentine soil (Sudarmono, 2007). The previous investigation indicated that a plant which could adapt to the condition of Kalimantan Selatan serpentine soil did symbiosis to develop mycorrhiza (Badruzsaufari et al*.*, 2013). A vast majority of plants are reported to be tolerant of the harsh characters of serpentine soil, which is helped by mycorrhizal symbiosis (Smith & Read,

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Corresponding Author: Witiyasti Imaningsih, e-mail: witiyasti.imaningsih@gmail.com

2008). Some fungi are specifically tolerant of toxic metals on serpentine soil (Gadd, 1993). The fungi, which are as ectomycorrhizal fungi, develop specific fruiting bodies that appeared at some seasons for their cycle life (Brundrett et al., 1996). It is included in the division of *Basidiomycota* and *Ascomycota* and also one genus in *Mucoromycotina*.

The mycorrhizal fungal diversity cannot be limited by Serpentine soils (Branco & Richard, 2010). In contrast, some arguments explain that it most likely affecting the toxic metals directly by enzymatic inhibition of fungal cellular (Gadd, 1993) and indirectly by free radical production (Dowling & Simmons, 2009). The comparison of fungal diversity among serpentine and non-serpentine soil (Branco & Richard, 2010) is reported as more diverse on serpentine soil and composed by the same fungal lineage. The highly diverse of ectomycorrhizal fungal communities in some extreme areas are always more classified from rare taxa (Taylor, 2002; Gardes & Bruns, 1996; Gehring et al., 1998). Accordingly, the ectomycorrhizal fungi originally from serpentine soil are unique and have a special mechanism to avoid the harsh serpentine soils.

The symbiosis of ectomycorrhizal fungi is specific (Brundrett et al., 1996). The genus of plant that capable of developing the association with ectomycorrhizal fungi was *Acacia* (Bellgard, 1991), Dipterocarps (Brundrett, 2009; Karmilasanti & Maharani 2016; Ulfa et al., 2018), *Pinus* (Koide & Wu, 2003; Darwo & Sugiarti, 2008; Dewi et al., 2016) and *Shorea* (Alamsjah & Husin, 2010; Ramadhani et al., 2018). Karmilasanti & Maharani (2016) also reported the host besides Dipterocarp, such as *Canarium*, *Cleodepas*, *Gluta*, *Krema*, *Madhuka*, *Ptarospermum*, and *Syzygium*. Several endemic serpentine soil plants such as jambu sakati (*Eugenia* sp.) and other woody plants, such as pines, usually grow on the serpentine soil of Taman Hutan Rakyat (Tahura) Mandiangin. Thus, the plant vegetation found on the soil suggested that it might associate with particular ectomycorrhizal fungi.

Materials and Methods

Site and time of sampling and experiment

The sample exploration to collect fruiting bodies of ectomycorrhizal fungi was conducted in January, March, April, May, and June 2014, which covered the rainy season. The exploration located in Taman Hutan Rakyat (TAHURA) Mandiangin, South Kalimantan Indonesia, where serpentine soil found. The sampling location was grown by woody plants, majorities such as Pine, *Eugenia,* or Sakati plant (local name), *Shorea*, *Acacia,* and *Leucaena leucocephala*. The experiment to identify the fungi was conducted at the microbiology laboratory and physiology/anatomy laboratory of the Basic Laboratory of Mathematics and Natural Science of Laboratory of Lambung Mangkurat University, from March until November 2014.

Mushrooms sampling

Mushrooms sampling was started with searching the fruiting bodies adjacent to the host plants (Darwo & Sugiarti, 2008). The mushrooms should be fruiting bodies that grow directly on the soil and near of woody plant. The condition and characters of fruiting bodies were recorded. The important characters that observed were fruiting body shape, cap shape, lamella shape, stem shape, and the existence of annulus ring and scales (Brundrett et al., 1996).

Identification of mushrooms

The genus name identification was based on Brundrett et al. (1996) and using the website http://mushroomexpert.com by Michael Kuo (2020). Then the genus status of fungi assumed as ectomycorrhizal fungi was identified on http://mycorrhizas.info by Mark C. Brundrett (2008) and some literature.

Results and Discussion

Mushrooms were found near the host plant. Ch1 was located near *Leucaena leucocephala*. Ch2, Ct, Rm1, and Rm2 were found near Sakati (local name) *Eugenia* sp. Ly was found near *Caesalpinia pulcherrima*. Tp was found near *Mangifera indica*. Their fruiting bodies were showed in figure 1. Macroscopic observation showed that specific forms for fungi indigenous South Kalimantan serpentine soil. They were in the types of puffball, coral fungi, fan-shaped, and toadstool. The result observation could be seen in table 1.

Chlorophyllum

Chlorophyllum is known as parasol fungi. The first stage before being a mature mushroom, it will be like parasol on the cap. After being mature, the cap will open and be wider to side. Sample Ch1 and Ch2 were identified as *Chorophyllum*. They had veil remnants on the cap and membranous annulus on the stem. The Ch1 was a young fruiting body of *Chlorophyllum*. All of the caps were in parabolic or parasol shape. The fruiting bodies were found making colonies (not too crowded) and distributing around the host plant. The Ch2 was the mature fruiting bodies of *Chlorophyllum*. The shape was in the umbonate. The cap was almost flat and wide with the single knop on the center of the cap. The colors of the Ch1 and Ch2 were cream of cap and stem, and sometimes the over mature fruiting body would show the blackish on the stem. The height was about 11,3 – 14,6 cm (Ch1) and 13,4 – 15,6 cm (Ch2). The diam. of the cap was about 5,5 – 5,9 cm (Ch1) and 7,9 – 9,5 cm (Ch2). Both of the samples had the membranous annulus.

Lycoperdon

Puffball is a unique mushroom that has a form like a bulb but sometimes squeezes texture. One genus in the Puffball form is a sample Ly. The Ly was looked like swell or bulge cap shape. Some of *Lycoperdon* were known as saprophytic fungi, but few of them were also ectomycorrhiza. The Ly had clearly cream color and free of the ornamentation on the surface of the fruiting body. The Ly was found only for one fruiting body. The texture was a squeeze, but exactly compact inside. The height was 7,6 cm. The stem part was tapering to base.

Cantharellus

The other unique mushroom was sample Ct formed like a trumpet with lamella that blooms on the top. The Ct was identified as *Cantharellus*. The Ct was found for two fruiting bodies. The cap was smooth of surface, wavy of margin, and free of veil remnant. The gill spacing had particularly distant. The cap was infundibuliform (7,3 – 7,5 cm).

Thelephora

Sample Tp was like a small and flattened fan identified as *Thelephora*. Different from other mushrooms as toadstool form, *Thelephora* had not lamella that blooms on the top, just flat for lamella or almost absent. The texture of the fruiting body was hard and rigid. They grow directly on the soil with colonies and crowded fruiting bodies. The color was white to brown. The cap was flattened skin-like (1,5 – 4,7 cm). The stem was short (0,7 – 1,0 cm) and directly grow on the soil.

Ramaria

Besides the toadstool and puffball, this study also found the coral fungi. Sample Rm1 and Rm2 had coral form fruiting bodies. The difference among other genera of *Ramaria* was the style of branching pattern on the tip of the fruiting body, color, and size (for morphology). The difference between Rm1 and Rm2 was the typical branching on the apex of the fruiting body. The Rm1 had the singular tip, but the Rm2 had the dichotomous branching (cloven to be two). The Rm1 had smooth stem (1,1 – 1,7 cm diam.), but the Rm2 had Smooth (1,2 – 1,6 cm diam.). The Rm1 had the 7 – 14 cm of height and the brownish color was clear of the whole fruiting body. The Rm2 had 6,6 – 7,3 cm of height, and the color was orange with white on the tip of the fruiting body. Both of the fruiting bodies were exactly different for the species based on their morphological characters. Exeter et al. (2006) classified many species in *Ramaria* based on the characteristics such as color or pigment of the fruiting body.

Figure 1 Mushrooms indigenous South Kalimantan serpentine soil. (a) *Chantarellus* Ct; (b) *Chlorophyllum* Ch1; (c) *Chlorophyllum* Ch2; (d) *Lycoperdon* Ly; (e) *Ramaria* Rm1; (f) *Ramaria* Rm2; (g) *Thelephora* Tp. Scale bars on the side of photos are in 5 cm.

Table 1. Macroscopic observation of the fruiting bodies

Sample code	Fruiting bodies morphology							Genus
	Shape of fruiting body	Cap shape (cap diam.)	Cap surface and margin	Gill spacing	Stem (stem diam.)	Height	Color	
Ct	Toadstool	Infundibuliform (7,3 – 7,5 cm)	Smooth and Wavy	Distant	Free of membranous annulus (0,6 – 1,3 cm)	7,6 – 8,1 cm	Cream	*Cantharellus*
Ch1	Toadstool	Parabolic (5,5 – 5,9 cm)	Universal veil remnant and eroded	Crowded	Exist of membranous annulus (0,7 – 1,3 cm)	11,3 – 14,6 cm	Cream	*Chlorophyllum*
Ch2	Toadstool	Umbonate (7,9 – 9,5 cm)	Universal veil remnant and eroded	Crowded	Exist of membranous annulus (0,8 – 1,4 cm)	13,4 – 15,6 cm	Cream	*Chlorophyllum*

Ly	Puffball	-	-	-	Smooth and tapering to base (2,7 – 3,2 cm)	7,6 cm	Cream	*Lycoperdon*
Rm1	Coral fungi	-	-	-	Smooth (1,1 – 1,7 cm)	7 – 14 cm	Brown	*Ramaria*
Rm2	Coral fungi	-	-	-	Smooth (1,2 – 1,6 cm)	6,6 – 7,3 cm	White to orange	*Ramaria*
Tp	Fan-shaped	Flattened skin-like (1,5 – 4,7 cm)	Smooth and wavy	-	Short (0,7 – 1,0 cm)	1,2 – 2,4 cm	White to brown	*Thelephora*

Mushrooms that assumed as ectomycorrhiza indigenous of South Kalimantan serpentine soil had been found in the *Basidiomycetes* division. Based on Brundrett et al. (1996), the mushrooms as ectomycorrhizal fungi were *Cantharellus*, *Lycoperdon*, *Ramaria*, and *Thelephora*. Then, one mushroom, *Chlorophyllum,* was not ectomycorrhizal fungi. *Chlorophyllum* was known as saprobic fungi (Kuo, 2020) and cannot make a symbiotic with plants. *Chlorohyllum* had been found on the grounds, lawns, and pastures in Australia (Vellinga, 2003) and China (Ge & Yang, 2006). The saprobic traits make the *Chlorophyllum* is capable of decomposing the substrate, such as litter on the ground. Some species of *Chlorophyllum* can also be harvested as edible mushrooms (Amadou et al*.,* 2009). Concernedly, this is dependent on where the mushroom grows. The mushrooms that are edible cannot be edible in the South Kalimantan serpentine soil. Some edible mushrooms can absorb and accumulate the mineral and toxic from the substrate or ground, such as *Lycoperdon perlatum* (Stihi et al., 2011). The fungus can absorb minerals that important for human diets, such as Cu, Cr, Fe, Mn, Se, and Zn, but also contain heavy metals as toxic, such as Cd, Ni, and Pb.

The heavy metals can be toxic to organisms, but some organisms have the mechanism to survive in that condition. Some fungi can survive in toxic environments because they can do biosorption by fungal melanin (Gadd & de Rome, 1988). The other mechanism also can be done by extracellular chelation (Bellion et al*.,* 2006). However, the ectomycorrhizal fungi that are surviving the plant root from toxic are played by filtering and protecting the root using mycelial mantle. This mechanism effects that the fruiting body will absorb the toxins (heavy metal). Therefore, the mushrooms found in this study may contain heavy metals in their fruiting bodies.

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