Journal of Biotechnology and Natural Science
Vol. 2, No. 1, June 2022, pp. 20-26
ISSN 2808-2508

Morphological Variation of "Jamur So" (*Scleroderma* sp.) from Purworejo Regency, Central Java

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ARTICLE INFO

Article history
Received July 5, 2022
Revised August 20, 2022
Accepted August 30, 2022

Keywords
Morphological variation
Scleroderma sp
Ectomycorrhyza
Purworejo

ABSTRACT

Fungal diversity is high in Indonesia, one of which is *Scleroderma* sp. Scleroderma forms a symbiotic relationship with *Gnetum gnemon*. Its fruiting body is puff-ball shaped, and its gleba can be consumed because of its taste and nutrition. Study on the *Scleroderma*'s diversity in Purworejo Regency has not been done. In this study, morphological characteristics were used as the main identification character. *Scleroderma* samples were collected from Grabag, Kemiri, and Kaligesing and then examined for their morphology and spore characteristics. Morphological traits were examined from fruit body shape (globose and kidney-shaped); and ornamentation on peridium layer (cracked, nodule, and peeling). Chemical content, temperature, and humidity of the soil were also examined as supporting data. Scleroderma fungi are found in three types based on the shape of the fruiting body, namely round, sub-globose and grooved like a kidney with an ornamentation of the peridium surface with nodules (warty), cracks, pyramidal protrusions and peeling. The spores are uniform with a spiny shape, varying in size between 5.70µm – 9.05µm with spore ornamentation, namely spiny reticulate and short spiny reticulate.

How to Cite: Pratiwi, A., Putri, D.A., Gultom, P.S., Indah, R., & Sasongko, H. (2022). Morphological Variation of "Jamur So" (*Scleroderma* sp.) from Purworejo Regency, Central Java. Journal of Biotechnology and Natural Science, 2(1),20-26.

1. Introduction

Fungi are very important organisms for soil, especially in forest ecosystems. Fungi support the continuity of the nutrient cycle chain that is important for maintaining soil fertility, while also contributing to building and maintaining soil structure, absorbing toxic materials (remediation), cycling carbon, nitrogen, phosphorus and sulfur, suppressing soil borne pathogens, promoting plant growth and affect vegetation. (Garbeva, et al., 2004; Singh & Singh, 2008).

Ectomycorrhizae are common on the roots of plants growing in cold and temperate climates, for example on shrubs in temperate climates, and on the roots of trees such as cypress, oak, pine, and eucalyptus. Ectomycorrhizae can originate from Basidiomycetes, Ascomycetes or Zygomycetes.

The ectomycorrhizal fungi develop mainly at the tips of the roots, then they penetrate into the cortex. In ectomycorrhizae, the fungus completely encloses each root branch in a hyphal sheath or blanket.

These hyphae only penetrate the intercellulars of the root cortex (Corryanti & Utomo, 2015). *Scleroderma* is an ectomycorrhizal fungus and or free saprotrophic fungus which is commonly found in the humus layer of soil, forest soil or in mutualistic symbiosis, one of which is with *melinjo* (gnetum) trees (Jeffries, 1999). The presence of this fungus will maintain the continuity of the material cycle in nature because of the ability to degrade macromolecules such as lignocellulose (Nasution et al., 2018).

Scleroderma is a macroscopic fungus commonly found in the tropics (Montagner et al., 2015) and belongs to the class of Gasteromycetes. *Scleroderma* has a fruiting body that covers its spores (spores in the fruiting body) which are globose or *spherical* in shape. *Scleroderma* is characterized by its thick and hard outer wall (peridium) with a mass of spores (gleba) which will become powder when mature/old and dispersed when the wall is broken/opened (Julich, 1988 in Kasiamdari et. al., 2003). Mature gleba is purple-black in color (Alexopoulos & Mims, 1979; Dwidjoseputro, 1978).The general characteristic of *Scleroderma* is that the fruiting body consists of a hard outer peridium with a purple-black gleba inside (Dwidjoseputro, 1987). Scleroderma varies in size, but usually larger fruiting bodies are found in the soil. *Scleroderma* is characterized by a thicker squamous peridium with a brownish color, and well-defined reticulated basidiospores (Nouhra et al. 2012).

Based on the information from the previous studies, *Scleroderma* fungus associated with a melinjo (gnetum) tree varies in morphological characters such as round fruiting body shape, like a kidney, and grooved, ornamentation of peridium surface is plain, cracked, noduled and peeled, surface of the spore is spiny and short spined, grooved and spiny grooved. This is in accordance with Chen et al. 2015, that Scleroderma has various fruiting body morphology. According to Kuo (2004), the surface ornamentation forms of Scleroderma spores that have been identified are spiny, or bumpy, not reticulated, and partially reticulated. The morphological features of *Scleroderma* basidiomas vary slightly, particularly in peridium thickness and spore size (Montagner et al., 2015).

Fungi grow well in fairly high humidity. Humidity range of 95-100% supports maximum growth in most mushrooms. Temperature range of 22°C – 28°C supports fungal growth well (Suriawiria, 2006). The air temperature is strongly influenced by the altitude, the higher is a place such as mountains, the lower is the air temperature and the lower a place, the higher is the air temperature in the area. Some of these factors may also affect the growth and development of the *Scleroderma* fungus.

Scleroderma is a fungus commonly found at the beginning of the rainy season in Java and is easily found in Purworejo Regency. Topographically, Purworejo Regency is a wet tropical climate with a temperature range of 19°C – 28°C, while the humidity is in the range of 70% - 90%. Environmental conditions in Purworejo Regency such as temperature, humidity and high rainfall allow for the varied characteristics of the *Scleroderma* fungus. No identification has been made for the species of *Scleroderma* found in Purworejo Regency.

2. Method

2.1. Tools and Materials

The tools used in this research were camera, ruler, plastic bag, knife, small shovel, loupe (magnifying lens), light microscope, optilab microscope, object glass, cover glass, tweezers, dropper.

The materials used in this study were fruit body samples of melinjo (gnetum) fungus *(Scleroderma* sp.), *Scleroderma* fungal spores, 70% alcohol, aquadest, glycerol, lactofenol, and 5% KOH.

2.2. Procedures

- Fungal fruiting bodies of *Scleroderma* were taken carefully from around melinjo trees in the Kaligesing (highland), Kemiri (lowland) and Grabag (coastal) sub-districts, photographed

and put in a plastic bag. Examination of fruit body morphology was carried out by observing fresh fruit bodies and taking notes of the fruit body shape, color, texture, peridium surface ornamentation, peridium thickness, and gleba color using the naked eye or using a loupe.

- The observation of Scleroderma fungal spores was carried out by heating the spores in 5% KOH in a test tube for each spore in each sample of the fruiting body of the old phase characterized by a non-spongy texture, black gleba color, like flour (semi-permanent preparations), then Spores in 5% KOH were taken using a loop needle, placed on a glass object, dripped with glycerol then added with lactofenol dye and observed under a light microscope with a magnification of 10x40. Observation of *Scleroderma* fungal hyphae was carried out by separating the hyphae in a mixture of glycerol and lactofenol dye using a brush and then observing them under a light microscope. The morphological characters of *Scleroderma* found in Purworejo Regency are described using reference by Kuo (2011).

3. Findings and Discussion

Sample taking of *Scleroderma* fungi was carried out in three districts in Purworejo Regency, namely Grabag, Kemiri, and Kaligesing. There were 15 samples of *Scleroderma* fungi collected in Purworejo with various characteristics. The number of samples taken from each of the districts also varied, with samples from Grabag (coastal area) being the most with as many as nine samples. This can be due to environmental factors such as temperature, humidity, and soil nutrition (Roosheroe et al., 2006). Grabag is a coastal area with lower temperature and higher humidity compared to Kemiri (low plain) and Kaligesing (plateau).

Table 1. Observation of environmental factors of the Scleroderma fungi sample taking

Parameter	Test results at each sampling site		
	Kaligesing	***Kemiri***	***Grabag***
pH	7,04	7,25	6,60
Moisture (%)	2,57	4,72	2,47
Air Temperature (°C)	31	31	31
Air Humidity (%)	67-73	70	70

Fungi can be found easily, especially in damp places, for example in litter and plants. Different media or substrates result in different types of growing fungi, so are the differences in environmental conditions, such as air humidity, soil moisture, temperature, soil acidity (pH), light intensity. Environmental factors greatly affect the growth of fungi, both mycelium and fungal fruiting bodies (Roosheroe et al., 2006). Environmental factors are also one of the supporting factors for the growth of macroscopic fungi. The results of the acidity (pH) measuring at each fungi collection site showed the range of 6.6-7. Macroscopic fungi can live and grow well in the pH range of 5.5-7.5 (Nasution et al., 2018). The concentration of pH on the substrate can affect the growth of fungi, although indirectly, but affects the availability of the necessary nutrients. Most fungi grow well at acidic to neutral pH. Humidity at each location was in the range of 63-73%. Water moisture causes fungal hyphae to spread over the substrate surface (Carlile & Watkinson, 1994). The availability of water in the environment around the fungi is very important in maintaining water content in cells and plays a role in nutrient transport. The temperature at the three locations was in the range of 31°C. Based on the temperature range obtained from the five locations, these temperatures still allow the fungus to grow. The maximum temperature for mushrooms to grow ranges from 30 to 40°C with an optimum temperature of 20–30°C (Carlile & Watkinson, 1994). The overall altitude of the locations ranges from 0 to 120 m above sea level. According to Warisno and Dahana (2010), altitude determines air temperature, intensity of sunlight and rainfall, which in turn affects plant growth, in which case will also have an impact on fungal growth. Light can stimulate or be an inhibiting factor for the formation of reproductive organ structures and spores in fungi.

The results of the morphological characterization of the Scleroderma fungus are presented in Table 2. The forms of ornamentation of the peridium or outer peridium of the Scleroderma fungus from Grabag District were warty (nodules), pyramidal protrusions, cracks and peels. The Scleroderma fungi samples from Kemiri District had the peridium with cracks and warty surface ornamentation, while the Scleroderma fungi samples from Kaligesing District had the peridium surface ornamentation of peels, cracks, and warty (pimples). Peridium surface ornamentation in the form of pyramidal protrusions was only found in Grabag District, while in Kemiri District there was no peel-shaped peridium surface ornamentation as found in Grabag and Kaligesing Districts.

Table 2. Results of observation of Scleroderma fungal morphology

Character	Morphological observation from each of sampling location		
	Grabag	***Kemiri***	***Kaligesing***
Fruiting body shape	Curved like kidney, sub globose and globose	sub globose and globose	Curved like kidney, sub globose and globose
Fruiting body color/outer (upper) peridium	Yellow-brown	Brown	Yellow brown-brown
Peridium surface ornamentation	Present	Present	Present
Ornamentation shape of peridium surface	Peel, crack, warty, pyramidal protrusion	Crack, warty	Peel, crack, warty
Peridium layer color/inner peridium	Yellow	Yellow	Yellow
Number of peridium layers	One layer	One layer	One layer
Peridium thickness	1:7 mm – 1,5: 33 mm	2:11 mm – 1,5:29 mm	2:13 mm – 4:211mm
Gleba color	Blackish purple, brown, white, black	Brown, blackish purple	Yellow, brown, blackish brown
Gleba chamber divider (present / absent)	Absent	Absent	Absent
Consistency	Fragile, hard	Hard	Fragile, hard
Growth habit	Solitary and clustered on ordinary, mossy, and grassy soil	Solitary on grassy soil	Solitary on ordinary, mossy, and grassy soil
Spore ornamentation shape	Spiny or bumpy not reticulate	Spiny or bumpy not reticulate	Spiny or bumpy not reticulate
Spora size	6,34-8,15µm	6,10-6,93 µm	5,70-9,05µm
Hyphae dividers	Present	Present	Present
Branching	Present	Present	Present
Clamp connections	Present	Present	Present

The peridium layer of the Scleroderma fungus fruiting body was found to have a thickness of 1-4 mm with a single layer of peridium and the color of the peridium layer was yellow. Peridium thickness of Scleroderma mushroom samples from Grabag and Kemiri Districts was 1-2 mm, while the ones from Kaligesing was 2-4 mm. This is probably due to the factors of altitude and air humidity as well as soil mineral content in Kaligesing District.

The shape diversity of Scleroderma fungal fruiting bodies found in Purworejo Regency was seen in three types of shape namely globose, sub-globose, and kidney-like fruiting bodies with ornamentation on the peridium surface in the forms of nodules (warty), cracks, pyramidal protrusions and peeling (Figure 1.).

Figure 1. Variations of *Scleroderma* peridium from Purworejo regency, a. fungus from plateau, b. fungus from coastal area and c. fungus from low plain

There were two samples of Scleroderma sp. with a globose fruiting body shape, they were the ones found in Grabag (coastal area) and Kalimeneng (lowlands). The samples of Scleroderma fungi with sub globose fruiting bodies were six samples from Grabag (coastal), one sample from Kalimeneng (lowlands), and one sample from Kaligesing (highlands). While the samples of Scleroderma mushrooms with curved fruit body like a kidney were two samples from Grabag (coastal), and three samples from Kaligesing (highlands). The fruiting bodies of ectomycorrhizal fungi were found growing on the soil surface and in the litter (Figures 2, 3, and 4).

Figure 2. *Scleroderma* sp. fungus from Grabag (a) fruiting body, (b) cross- section of fruiting body, (c) spore

Figure 3. *Scleroderma* sp. fungus from Kalimeneng (a) fruiting body, (b) cross-section of fruiting body, (c) spores

Figure 4. *Scleroderma* sp. fungus from Kaligesing (a) fruiting body, (b) cross-section of fruiting body, (c) spores

The radius where ectomycorrhizal fruiting bodies were found on the trunk of the host tree varied. The fungus with *puffball* shape belonging to the Gasteromycetes contained more spores than the umbrella-shaped ectomycorrhizae. The spore wall in the fruiting body was thicker and consequently more resistant to unfavorable environmental conditions (Anggraini, et al, 2015). The Scleroderma spores found in Purworejo Regency had a size of 5.70µm – 9.05µm with spore ornamentation in the shape of spiny reticulate and short spiny reticulate. The hyphae characteristic observed in Scleroderma was the presence of a clamp connection. Scleroderma mushroom samples from three districts in Purworejo Regency had clamp connections. Clamp connections serve to assist in the process of nuclear division in dikaryon-featured Basidiomycota fungi so that their daughter hyphae get two haploid nuclei.

4. Conclusion

Three types of Scleroderma fungi were found in Purworejo Regency which were distinguished based on the shape of the fruiting bodies, namely round (globose), sub-globose and grooved like a kidney with the peridium surface ornamentation with nodules (warty), cracks, pyramidal protrusions and peeling. The spores were uniform with spiny shape, varying in size between 5.70µm and 9.05µm with spore ornamentation of *spiny reticulate* and *short spiny reticulate*.

Acknowledgment

Highest appreciation is extended to the Ministry of Research, Technology, and Higher Education for their funding of this research, and LPPM UAD for their support and guidance to this research.

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