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Characterization of *Rhizoctonia*-like mycorrhizae associated with five *Dendrobium* species in Java, Indonesia

Abstract. Soelistijono R, Utami DS, Daryanti, Faizin M, Dian R. 2020. Plankton biodiversity in various typologies of inundation in Paminggir swamp, South Kalimantan, Indonesia on dry season. *Biodiversitas* 21: 1007-1011. This study aims to determine the morphological and anatomical characteristics of *Rhizoctonia*-like mycorrhizae associated with the roots of five *Dendrobium* species; to determine the association between *Rhizoctonia*-like mycorrhizae with the root of five *Dendrobium* sp.; to obtain difference between *Rhizoctonia*-like mycorrhizae with the other in adjacent location. Mycorrhizal observations of *Rhizoctonia*-like mycorrhizae in this study were carried out macroscopically (morphologically) and microscopically (anatomically). The macroscopic observation was performed by observing directly the development of fungal colonies on culture media. Microscopic observations were performed to determine the shape of the hyphal of fungi and the number of nuclei. The results showed that the *Rhizoctonia*-like mycorrhizae associated with the root of five species of *Dendrobium* sp. in Java were the binucleate *Rhizoctonia* groups (BNR). The binucleate *Rhizoctonia* has white colonies, right-angle branching hyphae, two nuclei, and brown hyphae. The association of the root of five species of *Dendrobium* sp. with *Rhizoctonia*-like mycorrhizae fungi was indicated by the existence of a peloton structure in cortical root tissue.

Keyword: *Dendrobium* species, *Rhizoctonia*-like mycorrhizae, peloton, Java

Orchid is one type of ornamental plant that has an attractive flower shape and color, is durable, and has a high selling value so that it is widely liked by the wider community (Widiastuti 2010). Apart from being an ornamental plant, some orchids have potential as medicinal plants, for example, *Spathoglottis plicata* as an ear medicine (Sadili 2011). Orchid belongs to the Orchidaceae family which consists of 800 genera and approximately 25,000 species. Based on the place of life, orchids are divided into 4 groups, namely epiphytes (which grow attached to trees), saprophytes (living in trash/humus), lithophytes (living in rocks), and terrestrial (which grows on the ground).

One of the most common epiphytic orchids is the genus *Dendrobium*. The *Dendrobium* genus is one of the largest orchid genus, which is ranked second after the genus *Bulbophyllum*. This genus has 600 species that spread of *Dendrobium* species (sp.) is very extensive and can be found from East Asia to the Pacific islands and the Himalayas through Myanmar to spread the Malay Peninsula, Australia, New Zealand, Papua New Guinea, China, Japan, Philippines, and Fiji (Northen 1986).

In nature, orchids need mycorrhizae to be able to grow, including *Dendrobium* sp. Because like the other orchids, *Dendrobium* sp. does not have food reserves (endosperm) for its development and growth. Withner (1959) mentioned

that orchid seeds have a very small form and do not have endosperm, so natural germination cannot occur. The absence of endosperm (food reserves) in orchid seeds is due to the absence of fusion between the sperm nucleus with the polar nucleus, or because degeneration always occurs after the union of a second sperm nucleus with two polar nuclei that produce a triploid nucleus. In some plants, before degeneration, the triploid nucleus still carries out several cleavages even though no perfect endosperm will be formed (Lenz and Wimber 1959).

In nature, *Dendrobium* seeds require mycorrhizae in supplying nutrients from the environment for germination (Smith and Read 2008). Through symbiotic relationships with plants, mycorrhizae play an important role in plant growth, disease protection, and overall soil quality improvement. Thus mycorrhizae play an important role in plant productivity (Siddiqui and Pitthel 2008). One of mycorrhizal fungi that is able to associate with the orchids is *Rhizoctonia* mycorrhizae (Athipunyakom and Manoch 2008). In addition to *Rhizoctonia* mycorrhizae, other fungi that can be symbiotic with the orchids are the genus *Epulorrhiza*, *Moniliopsis*, and *Ceratorhiza* (Smith and Read 2008). *Rhizoctonia* mycorrhizae are one of the *Rhizoctonia* species which has the ability to associate with orchid plants (Hayakawa et al. 1999). *Rhizoctonia* species consist of 3 groups depending on the number of nuclei. The first group is *Rhizoctonia* species, which has only 1 nucleus, this group is non-pathogenic. The second group of *Rhizoctonia*

species has 2 nuclei, are mycorrhizal in orchids, and the third group has many nuclei, are pathogenic like *Rhizoctonia solani* (Sneh et al. 1991).

Rhizoctonia mycorrhizae are capable of symbiosis with orchid root tissue and form hyphae twists that attach to the root cortex tissue in orchids. The association of *Rhizoctonia* mycorrhizae and orchid plants occurs at the stage of embryonic development, then the formation of shoots and roots is known as the stages of protocorm formation. Hyphae from *Rhizoctonia* mycorrhizae will penetrate orchid seed cell walls and then spread throughout other cell tissues. After the protocorm develops into a perfect plant (plantlet) then *Rhizoctonia* mycorrhizae hyphae tissue will be in the cortex of the orchid root and form a peloton structure (Senthilkumar et al. 2001).

Peloton is intracellular hyphae which in the cortical root tissue and usually only exist in a certain period before then undergoing lysis. Peloton is an accumulation of organic materials including protein, glycogen, and fat, which is the result of absorption of nutrients from the soil. When it is needed, the orchid embryo will absorb these organic materials for growth so that the peloton will undergo lysis (George 2008). Peloton is formed from binucleate *Rhizoctonia* (BNR) hyphae which penetrate and then infect the epidermal cells, then form a solid coil in the root (Brundrett 2004). Infection and peloton lysis occur repeatedly in cells. In orchid tissue culture, peloton will never form in seedling, because there is no association between *Rhizoctonia* mycorrhizae with orchid seeds. Because orchid seeds grow in the bottle (in vitro), it gets the supply of nutrients directly from growth media such as Murashige and Skoog or Vaccine and Went media.

There are several mycorrhizal fungi associated with *Dendrobium* sp. such as *Mucor* and *Rhizoctonia* (Mufidah et al. 2017.), however, there is no research on comparisons between *Rhizoctonia*-like mycorrhizae associated with *Dendrobium* the others in Central Java, Special Region of Yogyakarta, and East Java. Therefore, this study is

expected to obtain comparative data on various types of *Rhizoctonia*-like mycorrhizae that are located close to each other.

The research was carried out from March 2018 to April 2019 in the Microbiology Laboratory of the Faculty of Agriculture, Tunas Pembangunan University, Surakarta, Indonesia. Several samples of *Dendrobium* sp. were collected from Java Island, Indonesia, i.e. *Dendrobium lasianthera* from Kaliurang, Yogyakarta (800 m above sea level/m. asl), *Dendrobium lineale* blue from Sleman, Yogyakarta (300 m. asl), *Dendrobium aphyllum* from Surakarta, Central Java (100 m. asl), *Dendrobium fimbriatum* from Batu, East Java (1000 m. asl), and *Dendrobium phalaenopsis* from Malang, East Java (500 m. asl). All of the collected *Dendrobium*s are epiphytes on trees.

Isolation and identification of *Rhizoctonia*-like mycorrhizae

Isolation of *Rhizoctonia*-like mycorrhizae was carried out by cutting the tip of the roots of healthy *Dendrobium* sp. with a thickness of 3 mm in the transverse section. Root segments were placed on potato dextrose agar (PDA) in the Petri dish. Sample sterilization was not carried out on *Rhizoctonia*-like mycorrhizae isolation because it can kill mycorrhizal fungi. So that more than one fungi or bacteria colonies in medium. From several fungi or bacteria colonies obtain isolation was carried out fungi colonies suspected *Rhizoctonia*-like mycorrhizae. The growing fungi on PDA were purified to obtain pure isolate of *Rhizoctonia*-like mycorrhizae with the criteria of white colony fungi (Soelistijono 2011).

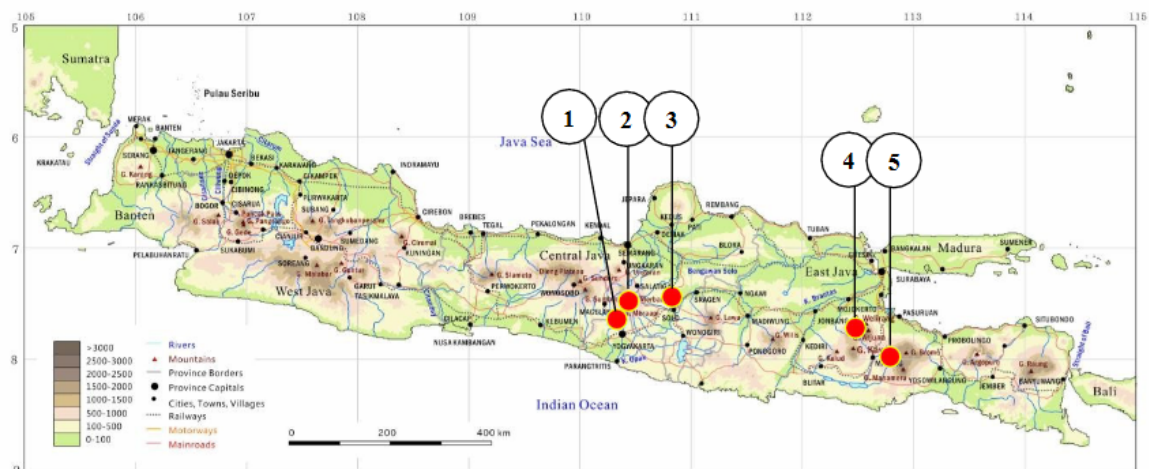


Figure 1. Sampling locations of *Dendrobium* sp. In Java, Indonesia. 1. Sleman, 2. Kaliurang, 3. Surakarta, 4. Batu, 5. Malang

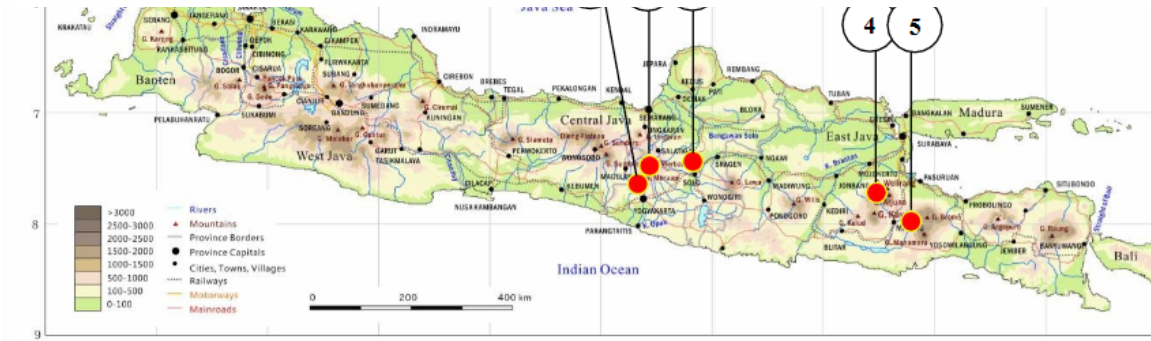


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Identification of *Rhizoctonia*-like mycorrhizae was carried out according to Barnett and Hunter (1972). Morphological characteristics were observed, i.e. colony color, branching shape, and the number of nuclei in the cell; while microscopic observation (anatomical) of hyphae structure (peloton) was done under XSZ 107 binocular microscope BN/Oregon/Yazumi at 400 times magnification. Observation of peloton structure was carried out microscopically by observing the presence of hyphal coil in the root tissue based on the method of Senthilkumar et al. (2001) which has been modified for safranin staining. Preparations were made by making cross-sectioned roots of the five species of *Dendrobium* sp. as thin as possible. Root pieces are placed on the glass object and then put on the glazed object with drops of safranin paint and also 90% alcohol.

Diversity of *Rhizoctonia*-like mycorrhizae associated with *Dendrobium* sp.

A total of 17 *Rhizoctonia*-like mycorrhizae were isolated from *Dendrobium* sp. Observations of fungal colonies on PDA showed that fungi have a white color. According to Athipunyakom and Manoch (2008), The colony of *Rhizoctonia* mycorrhizae in orchids are generally white. Macroscopic observations showed that fungal colonies growing on PDA was almost cover the surface of the Petri dish. The observations on the 17 *Rhizoctonia*-like mycorrhizae from *Dendrobium* showed that mycorrhizae from *D. lasianthera* have white (isolates number 1 and 2) and light brown colonies (isolates number 3). Mycorrhizal fungi from *D. lineale* has a light brown (isolate number 4) and white (isolate number 5, 6, and 7) colony, isolates from *D. aphyllum* has white colony (isolates number 8, 9, and 10), those from *D. fimbriatum* have white (isolate number 11, 13 and 14), and light brown colony (isolate number 12), while isolates from *D. phalaenopsis* have white colony (isolates number 15, 15, and 17). Almost all isolates were 2 nucleated (binucleate). Color differences in the same isolate can occur due to differences in the sporangium maturity. Sporangium maturity affects the color of sporangium from white, brown, and dark. The dark color indicates mature sporangium and is ready to spore out before finally forming sclerotium. Therefore, differences in colony's color can not be used as the main factor to differentiate mycorrhizae isolated from various species of *Dendrobium*. The identification should be based on the mycelium structure. The mycelium identification was carried out based on Barnett and Hunter (1972) i.e.: (i) colony color, (ii) number of nuclei, and (iii) shape.

Identification of *Rhizoctonia*-like mycorrhizae

Figure 2 shows that each isolate has white hyphae and the branching occurs at right angles. Observation shows that the main hyphae are longer and larger in diameter. Small hyphae are branched hyphae from the main hyphae. This is in agreement with Agrios (2005) and Sneh et al. (1991), that hyphae branches on *Rhizoctonia* mycorrhizae form angular angles and have brown pigment. The study of

Athipunyakom and Manoch (2008), showed that 7 isolates of *Rhizoctonia* mycorrhizae from *Spathoglottis plicata* from various places in Thailand had white, brownish-white or light brown colonies. The study of Soelistijono et al., (2011) also showed that 4 isolates of *Rhizoctonia* mycorrhizae from *Spathoglottis plicata* from various places in Java had white colonies. The study by Agustini et al., (2009) in the Jayapura Cycloops botanical garden showed that 10 isolates of mycorrhizae from orchid have different and various colors from white to black. Therefore, the color of the colonies of *Rhizoctonia*-like mycorrhiza is not always white. This is in agreement with Soelistijono (2011) that the colonies of *Rhizoctonia*-like mycorrhizae differ depending on their groups (isolates grouping).

Sneh et al., (1991) revealed that *Rhizoctonia* mycorrhizal isolates were included in the binucleate group. *Rhizoctonia*-like mycorrhizae collected in this study showed that their hyphae cells have two nuclei in each septum, therefore, these isolates can be classified as Binucleate *Rhizoctonia*-like mycorrhizae (BNR) which have two nuclei. Based on morphological and anatomical characteristics it can be concluded that all of the isolated fungi were *Rhizoctonia*-like mycorrhizae.

Peloton structure

Roots of *Dendrobium* sp. showed the hyphal coil structure in the root cortex as a result of *Rhizoctonia*-like mycorrhizae association with the root cortex. The study by Zumri et al., (2016) showed the formation of a peloton structure that occurred in the root cortex of *Vanda tricolor* seedling as a result of the association with *Rhizoctonia* mycorrhizae as a coil in the space between cells. Cross-section of the orchid root showed that peloton only presence in the cortical tissue, but not found in the other root tissues such as an epidermal or central cylinder. Orchid roots do not have cambium so the nutrient transport is spread outside. The presence of peloton in the root cortex tissue as the food reserves so that nutrients in the peloton can be absorbed and circulated followed by the lysis of peloton.

Smith and Read (2008) stated that *Rhizoctonia* mycorrhizae are capable of symbiosis with orchid root tissue and form hyphal coils that clump in the cortical root tissue. The nutrient absorption mechanism in the form of peloton structure shows that mycelium of *Rhizoctonia* mycorrhizae can penetrate the epidermal tissue and root cortex. In the cortex, mycelium enters the intercellular space and form a peloton. Inside the peloton, there is the accumulation of organic materials includes proteins, glycogen and fat and nutrients produced by absorption from the soil. Orchid embryos absorb these organic materials for their growth after that the peloton undergoing lysis (Soelistijono 2015).

Orchids have various heterotrophic levels. Low heterotrophic orchids require the presence of mycorrhizae in obtaining carbohydrates and other organic materials. Nutrient compounds provided by mycorrhizal fungi include sugar, vitamins, amino acids and other compounds that have not been identified. *Rhizoctonia* sp. produces niacin that can accelerate the germination and growth of orchid seeds.

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It is expected that *Rhizoctonia*-like mycorrhizae isolated in this study can be inoculated into orchids seedling from in vitro propagation (tissue culture propagation) so that the seedling will be able to grow better

compared to those not inoculated with *Rhizoctonia*-like mycorrhizae, and their growth is similar to orchid species cultivated in nature.

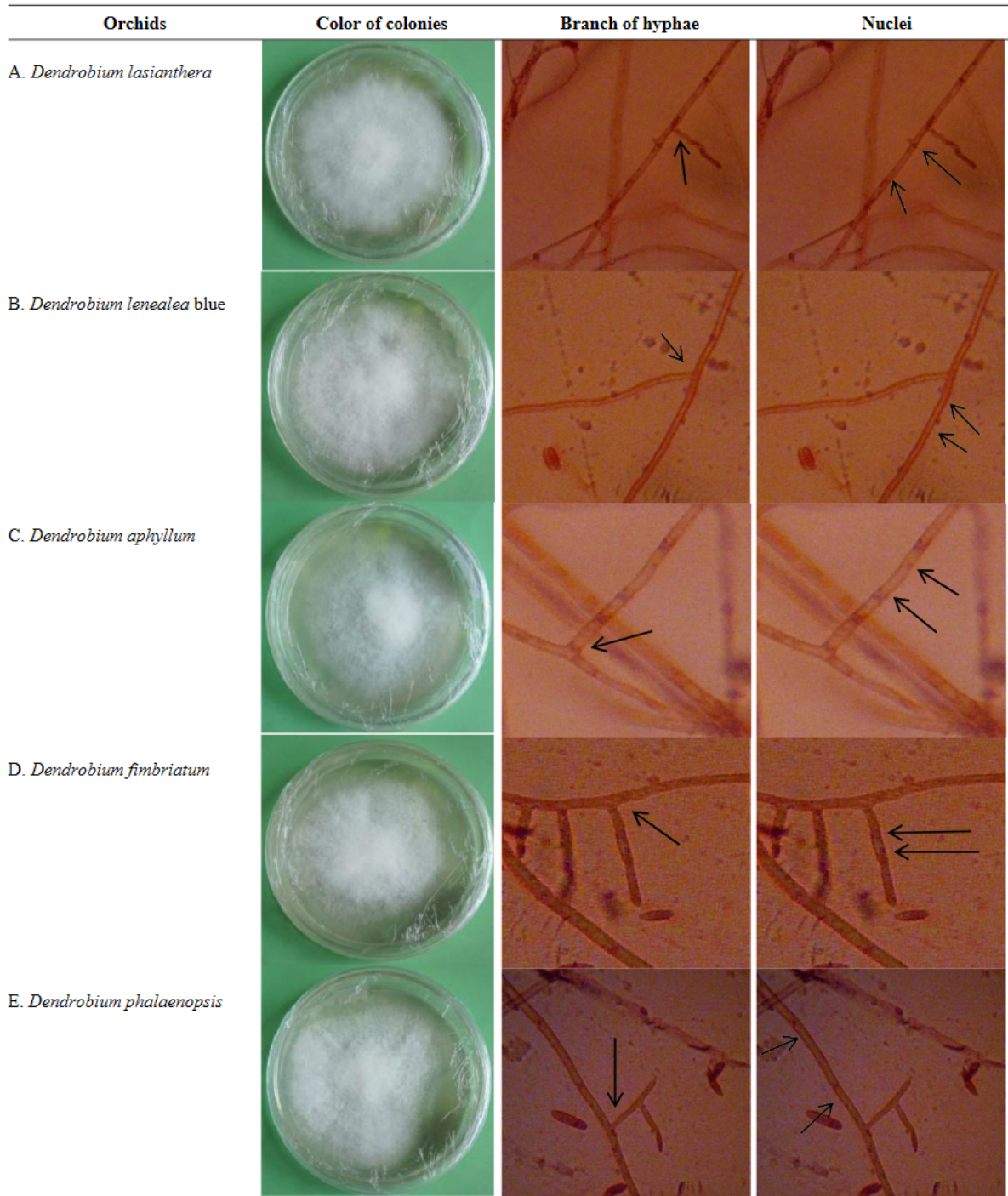


Figure 2. *Rhizoctonia*-like mycorrhizae colony from five *Dendrobium* species in Java, Indonesia. *Rhizoctonia*-like mycorrhizae colonies from: A. *Dendrobium lasianthera*, B. *Dendrobium linealea* blue, C. *Dendrobium aphyllum*, D. *Dendrobium fimbriatum*, E. *Dendrobium phalaenopsis*

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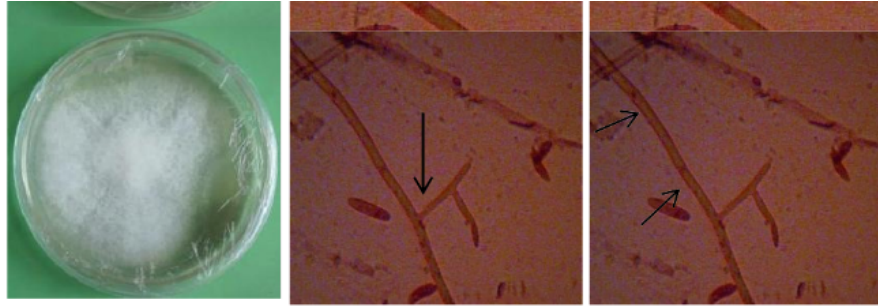


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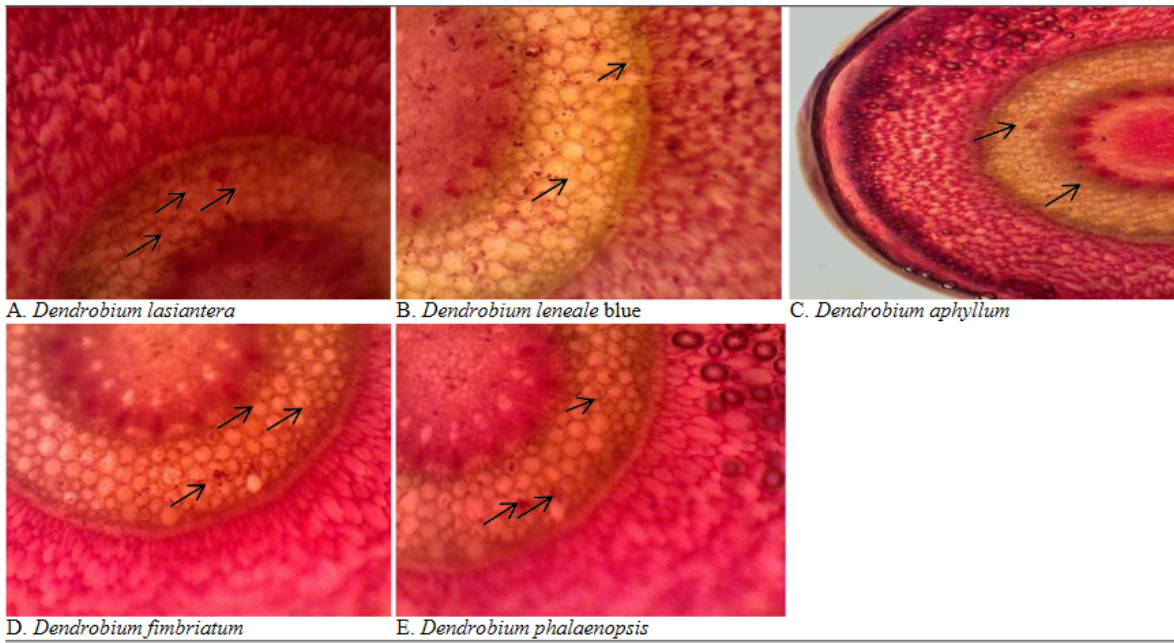


Figure 3. Peloton structure of *Rhizoctonia*-like mycorrhizae from: A. *Dendrobium lasianthera*, B. *Dendrobium linealea blue*, C. *Dendrobium aphyllum*, D. *Dendrobium fimbriatum*, and E. *Dendrobium phalaenopsis*

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