Open Science and Technology
Vol. 03 No. 02, 2023 (105-116)
ISSN (Print) :2776-169X
ISSN (Online) :2776-1681

Isolation and Identification of Arbuscular Mycorrhizal Fungi (AMF) Microscopically in the Rhizosphere of Peanut Plants

Isolasi dan Identifikasi Fungi Mikoriza Arbuskular (FMA) secara Mikroskopis pada Rhizosfer Tanaman Kacang Tanah

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Submitted:22 January 2024 Accepted: 31 January 2024 Published: 03 February 2024

ABSTRAK

Fungi Mikoriza Arbuskular (FMA) merupakan jamur yang bersimbiosis dengan perakaran tumbuhan tingkat tinggi. FMA dapat berperan sebagai agen biokontrol dan biofertilizer. Studi ini bertujuan mengetahui jenis-jenis FMA pada rhizosfer tanaman kacang tanah di Nagari Sawah Tangah, Kecamatan Pariangan, Kabupaten Tanah Datar dan sebagai tahapan awal dalam pemanfaatan FMA sebagai agen biokontrol dan biofertilizer. Sebanyak lima sampel tanah diambil menggunakan teknik *purposive random sampling*. Sampel tanah yang diperoleh disaring menggunakan teknik penyaringan basah. Identifikasi spora FMA dilakukan hingga tingkat genus berdasarkan karakteristik morfologi, yakni bentuk, warna, serta ornamentasi spora. Hasilnya menunjukkan bahwa jumlah spora FMA tertinggi pada rhizosfer tanaman kacang di Nagari Sawah Tangah, Kecamatan Pariangan, Kabupaten Tanah Datar adalah dari genus *Glomus* (3 tipe), yakni *Glomus* sp-1 sebanyak 15 spora, *Glomus* sp-2 sebanyak 12 spora, dan G*lomus* sp-3 sebanyak 74 spora (total 101 spora). Jumlah tertinggi kedua adalah dari genus *Acaulospora* (1 tipe) sebanyak 27 spora, sementara jumlah spora FMA terendah adalah dari genus *Gigaspora* (1 tipe) sebanyak 9 spora per 100 gr sampel tanah.

Kata kunci: *Acaulospora*, Fungi Mikoriza Arbuskular, *Gigaspora*, *Glomus*

ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) are fungi that have a symbiotic relationship with the roots of higher plants. AMF can act both as biocontrol agent and biofertilizer. This study aims to determine the types of AMF in the rhizosphere of peanut plants in Nagari Sawah Tangah, Pariangan Subdistrict, Tanah Datar Regency, as an initial stage in the application of AMF as a biocontrol agent and biofertilizer. Five soil samples were taken using a purposive random sampling technique. The soil samples obtained were then filtered using a wet filtration technique. Identification of AMF spores was carried out at the genus level based on their morphological characteristics, namely shape, color, and spore ornamentation. The results reveal that the highest number of AMF spores in the rhizosphere of peanut plants in Nagari Sawah Tangah, Pariangan Subdistrict, Tanah Datar Regency is from the genus Glomus *(3 types), namely* Glomus *sp-1 with 15 spores,* Glomus *sp-2 with 12 spores, and* Glomus *sp-3 with 74 spores (a total of 101 spores). The second highest number is from the genus* Acaulospora *(1 type) with 27 spores, and the lowest number of AMF spores is from the genus* Gigaspora *(1 type) with 9 spores per 100 gr of soil sample.*

Keywords: Acaulospora, *Arbuscular Mycorrhizal Fungi*, Gigaspora, Glomus

Open Science and Technology
Vol. 03 No. 02, 2023 (105-116)
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INTRODUCTION

Mycorrhizae, particularly Arbuscular Mycorrhizal Fungi (AMF), is a form of mutualistic symbiotic relationship between certain fungi and the roots of higher plants, usually vascular plants. This symbiosis occurs as a mutually beneficial relationship, where the fungi obtain carbohydrates and other elements for their growth (about 10%) from the host plant, while the fungi benefit the host plant by helping the plant absorb the essential nutrients, especially P element. Besides, AMF plays a role in increasing plant resistance to drought and pathogen infections, and helps in accelerating plant growth rates (Akhtar & Siddiqui, 2008; Smith & Read, 2008; Talanca, 2010).

AMF can trigger lignification in the root endodermis cells to form a barrier against pathogen penetration. AMF in plants also plays a role in triggering competition among pathogens for obtaining carbon compounds so as to inhibit the growth of pathogens in plants (Akhtar & Siddiqui, 2008; Talanca, 2010). The induction of AMF into plants can also promote systemic resistance that can positively affect plant physiological and biochemical responses through the increased production of chemical compounds, such as jasmonic acid, ethylene, chitinase, phytoalexins, and salicylic acid. Salicylic acid is one of the compounds that induce the formation of pathogen related protein (PR-protein) and plays a role as a signal molecule to induce plant systemic resistance (Chen et al., 2010; Vlot et al., 2009).

The mechanism of AMF as a biocontrol agent in plants can be direct or indirect. Direct mechanisms can include competition for obtaining nutrients and growth space, while indirect mechanisms can include induction of systemic resistance, changes in root morphology, and physiological and biochemical changes (Pozo et al. 2009; Prasasti et al. 2013; Vlot et al., 2009).

For cultivated plants that are susceptible to pathogens and disease infection, one type of biological control mechanism that has the potential to be developed is resistance induction (immunization), because in addition to being practical, economical, and efficient, this mechanism is also more environmentally friendly (Habazar, 2001 cit. Sulyanti, 2012). Resistance induction is a process of stimulating host plant resistance to pathogens without the introduction of new genes. In particular, the introduction of AMF can positively affect the physiological and biochemical responses of plants through the increased production of several secondary metabolite compounds, such as ethylene, chitinase, phytoalexins, jasmonic acid, and salicylic acid, which can increase plant resistance to pathogen infection (Pozo et al., 2009; Vlot et al., 2009).

Information regarding the types of AMF and their utilization in peanut plants, especially in Nagari Sawah Tangah, has never been reported so far. Therefore, this study aims to determine the types of AMF in the rhizosphere of peanut plants in Nagari Sawah Tangah, Pariangan Subdistrict, Tanah Datar Regency. This study was also conducted as an initial stage in the utilization of AMF as a biocontrol agent and biofertilizer. This study is expected to be able to identify indigenous AMF isolates from the rhizosphere of peanut plants in Nagari Sawah Tangah. As a future implementation from this, future studies can be carried out to investigate the compatibility and effectiveness of AMF species found to be able to suppress pathogen attacks that cause disease so as to increase the growth of peanut plants.

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MATERIALS AND METHODS

This study was conducted from July to October 2019. Sampling was carried out at the peanut plantation at Nagari Sawah Tangah, Pariangan Subdistrict, Tanah Datar Regency. The samples obtained were then tested at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University.

Research Implementation

Soil and root sampling

Soil and root samples of peanut plants were collected at Nagari Sawah Tangah, Tanah Datar Regency (Figure 1). A total of five soil samples were taken using purposive random sampling technique, then the soil were composited and filtered using a wet filtration technique. First, prior to soil sampling, a measurement were taken at 0–10 cm from the plant stem, then at a distance of 10 cm from the plant, soil sampling was carried out at a depth of 0–20 cm from the soil surface. The roots of the plants and the surrounding soil were then collected using a shovel or knife as needed. The roots at the neck were cut, then put into a plastic bag along with the soil sample. The plastic bag was left open for a while so that the soil sample was cool enough so as to reduce its respiration rate. The soil and root samples were then processed immediately upon arrival at the laboratory, or put in the refrigerator for a while if they could not be processed immediately (Nusantara et al., 2015).

Figure 1. Soil sampling at Nagari Sawah Tangah, Tanah Datar Regency. A. Sampling location, B. Soil sample.

Spore extraction

The spore extraction process (Figure 2) was carried out as follows. Soil samples and roots were weighed (100 gr), then water was added (4 ml/1 gr of soil). The soil and root suspension was crushed using a blender for 1 minute. Next, the soil suspension and roots were poured into a multilevel filter. The top part is the filter with the largest mesh size (500 µm) and the bottom part is the filter with the smallest mesh size (38 µm). The resulting precipitate at the bottom of the filter (smallest hole size as well as in the reservoir) was transferred into a glass goblet with the addition of water from a spray bottle. The suspension was then stirred and poured into a centrifuge tube. The soil suspension was mixed with 60% of sugar solution twice the suspension volume, then

centrifuged at 3,000 rpm for approximately 3 minutes. The spores will float on the sugar solution or at the top of the clear-textured suspension. Next, the clear suspension was poured onto a filter surface that has been covered with filter paper and cleaned using running water to avoid spore lysis. Next, the spores were transferred to petri dishes (Nusantara et al., 2015; Setiawan et al., 2014). Finally, the AMF spores were observed using a stereo microscope and categorized based on their color, size, and shape.

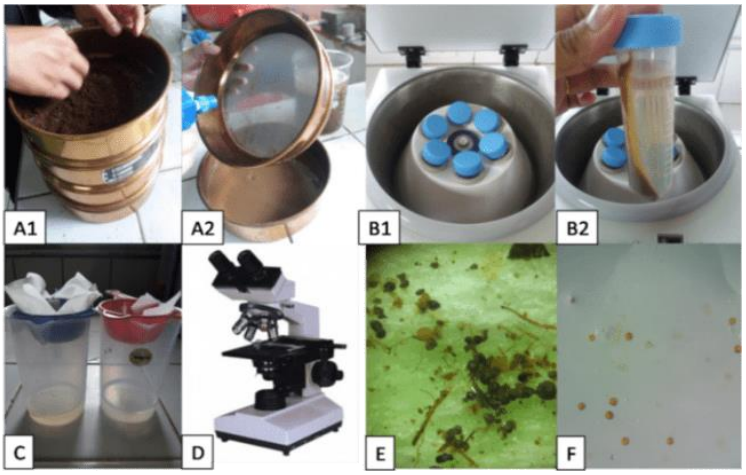

Figure 2. Extraction of AMF spores from soil samples along with peanut roots. A1–A2. Screening of soil samples; B1–B2. Soil samples were centrifuged at 3,000 rpm for 3 minutes; C. The centrifugation results were filtered; D. The filter results were observed using a microscope; E. Image of spores observed under a microscope; F. Grouping of AMF based on shape, color, and size.

Spore population count

The filtered spores were grouped on the petri dishes. The spore population was counted using a 1 cm x 1 cm of grid line made on a piece of paper that was placed under the petri dish. The spore population was counted using a microscope in each field of view until all spores at the petri dishes were observed (Nusantara et al., 2015).

Identification of indigenous Arbuscular Mycorrhizal Fungi (AMF)

AMF spores were observed using a binocular microscope. First, the object glass was prepared: the left side was dripped with PVLG solution and the right side was dripped with Melzer solution. Similar spores were placed on each drop of the solution, then each part was covered with a cover glass. The spores were broken by pressing the surface of the cover glass using a toothpick. The object glass was then placed under the objective lenses of the microscope to observe the spore's reaction with Melzer solution (Nusantara et al., 2015). The color change of spore in Melzer solution is one of the indicators to determine the type of spore present in the soil sample. Identification and characterization of various AMF isolates were carried out up to genus level based on the guidance and classification established by INVAM (2024).

Observation

Open Science and Technology
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ISSN (Print) :2776-169X
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The AMF spores were observed under a stereo microscope and grouped based on their morphological characteristics (size, color, cell wall layer, ornamentation, and shape of hyphae attached to the spore wall). Next, the number of AMF spores in each genus and species were counted and expressed in units per 100 gr of soil sample observed.

RESULTS AND DISCUSSION

The Number of Arbuscular Mycorrhizal Fungi (AMF) Spores

The genus *Glomus* has the highest number of AMF spores, namely 101 spores, while the genus *Gigaspora* has the lowest number of AMF spores, namely 9 spores per 100 gr of soil sample (Table 1). The microscopic features of each type of the spore obtained are presented in Figures 3-7.

Table 1. The number of AMF spores per 100 gr of soil sample.

No.	Genus	Spore Type of AMF	Amount	Number of Spores per Genus
1.	*Glomus*	*Glomus* sp-1	15	
		Glomus sp-2	12	101
		Glomus sp-3	74	
2.	*Acaulospora*	*Acaulospora* sp.	27	27
3.	*Gigaspora*	*Gigaspora* sp.	9	9
		Total		137

Arbuscular mycorrhizal fungi (AMF) are present in almost all natural terrestrial communities and form symbiotic relationships with more than 80% of plant species. The genus *Glomus* has the widest distribution and is the most tolerant towards soil salinity conditions. The large population of *Glomus* spores may also be due to the fact that *Glomus*-type AMF has a greater number of species in nature compared to that of other genus (Smith & Read, 2008; Delvian, 2006).

The large number of spores from the genus *Glomus* found in this study is thought to be due to the fact that the sampling location is a former rice field that has clay-textured soil so that it is naturally preferred by the genus *Glomus*, which has spores that are smaller than that of other genus. In addition, the genus *Glomus* is more able to adapt to wide range of environment condition. This is shown by several previous studies, such as the results by Setiadi and Setiawan (2011) who isolated AMF from post-nickel mining rehabilitation areas, Manaroinsong and Lolong (2015) from oil palm plantation area, and Anggreiny et al. (2017) from the tin mining land revegetation area. All of which obtained the results that the genus *Glomus* had the highest population compared to that of other genus at those areas. In addition, Mathimaran et al. (2005) also stated that AMF from the genus *Glomus* was able to survive and thrive in a wide range of soil textures, even in soils with clay textures.

Identification of Arbuscular Mycorrhizal Fungi (AMF)

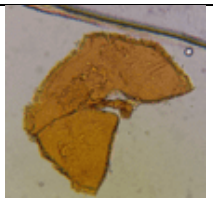
The spore identification from soil samples at Nagari Sawah Tangah, Tanah Datar Regency resulted in three genus of AMF, namely *Glomus* (3 types), *Acaulospora* (1

type), and *Gigaspora* (1 type) (Table 2). The AMF species identified from the rhizosphere of peanut plants are described as follows: Spore of *Glomus* sp-1 is characterized by round shape, comprises three layers of spore wall where the outer spore wall has been partially peeled off, brownish yellow in color, and did not react with Melzer solution. Spore of *Glomus* sp-2 is characterized by spherical shape, comprises two layers of spore wall, dark brown in color, and did not react with Melzer solution. Spore of *Glomus* sp-3 is characterized by spherical shape, comprises three layers of spore wall, has substanding hyphae, yellow in color, and did not react with Melzer solution. These results are in accordance with INVAM (2024), which stated that spore development in the genus *Glomus* occurs through blastic expansion of hyphal tips. The outer layers of the spore wall often peel off as the spore ages (both in soil and in pot culture storage). Developmentally, these layers are the first components of the spore wall to form in juvenile spores. In several species, the hyphae of mature spores are so thin that they are quite difficult to be observed or separated from the spore.

Spore of the genus *Acaulospora* is characterized by round shape, brown in color, comprises three layers of spore wall, the outer layer did not react with Melzer solution, and the inner layer reacted with Melzer solution. This result is in accordance with Nusantara et al. (2015), which stated that in the spores of the genus *Acaulospora*, the outer layer did not react with Melzer solution, the inner layer reacted with Melzer solution (produced darker color to purplish red), and had various ornaments depending on the species.

The spore of the genus *Gigaspora* is characterized by large and perfectly round shape, bright yellow in color, smooth and thin layer of spore wall, its hyphae form the bulbous suspensors, and reacted with Melzer solution thoroughly. This result is in accordance with Asmarahman et al. (2018), who identified AMF from mining land. They also obtained AMF from the genus *Gigaspora* with the following characteristics: the size of the spores varied, namely 225 x 216 µm and 357.5 x 357 µm, yellow and dark red in color, thin layer of spore wall (± 2 layers), reacted with Melzer solution thoroughly, and had a bulbous suspensor. INVAM (2024) stated that the process of *Gigaspora* development occurs indirectly from hyphae. Initially, the end of the hyphae (substanding hyphae) is rounded, which is called bulbous suspensor. At the top of this bulbous suspensor, a small sphere appears, which gets bigger and bigger until it reaches the maximum size, which eventually becomes a spore known as azygospora. Besides, a distinctive characteristic worth mention here is that in the genus *Gigaspora*, there are bulbous suspensors without germination sheld.

Table 2. Spore morphological characterictics of each of AMF species.

Species	Spore Morphological Characteristics	Reaction with Melzer Solution	Image
Glomus sp-1	Spore is round, comprises three layers of spore wall, the outer spore wall is partially peeled off, brownish yellow in color, and did not react with Melzer solution.	Did not react	

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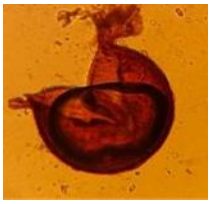
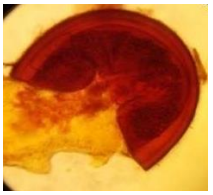
Glomus sp-2	Spore is spherical, comprises two layers of spore wall, dark brown in color, and did not react with Melzer solution.	Did not react	
Glomus sp-3	Spore is spherical, comprises three layers of spore wall, has substanding hyphae, yellow in color, and did not react with Melzer solution.	Did not react	
Acaulospora sp.	Spore is spherical, brown in color, comprises three layers of spore wall, the outer layer did not react with Melzer solution, and the inner layer reacted with Melzer solution.	Partially reacted	
Gigaspora sp.	Spore is large and perfectly round, bright yellow in color, spore wall is smooth and thin, hyphae forms bulbous suspensors, reacted with Melzer solution thoroughly.	Thoroughly reacted	

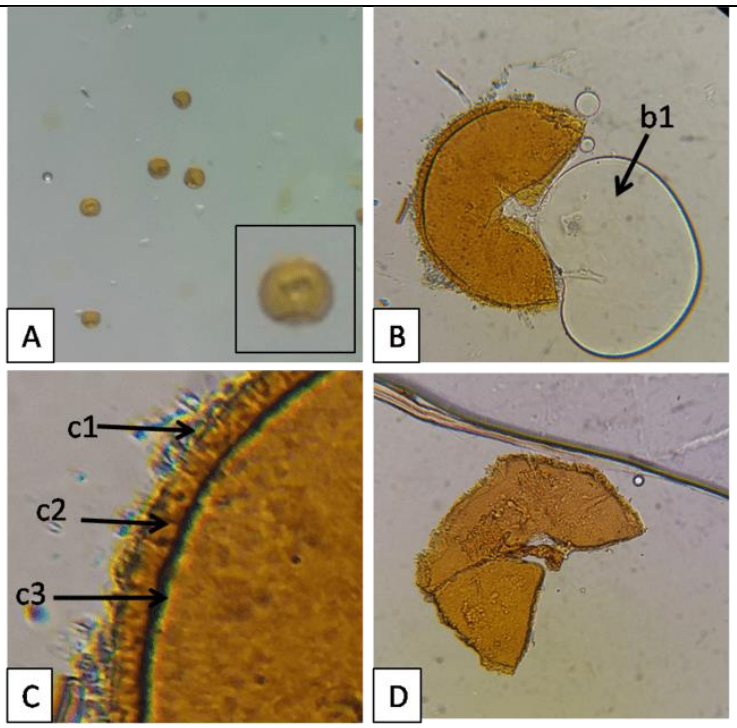


Figure 3. AMF of *Glomus* sp-1. A. Spores of Glomus sp-1 on stereo microscope (40 x magnification), B. Spore of *Glomus* sp-1 on PVLG solution (400 x

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magnification) (b1. lipid), C. Spore wall of *Glomus* sp-1 (c1. first layer of spore wall, c2. second layer of spore wall, c3. third layer of spore wall), D. Spore of *Glomus* sp-1 on Melzer solution (400 x magnification).

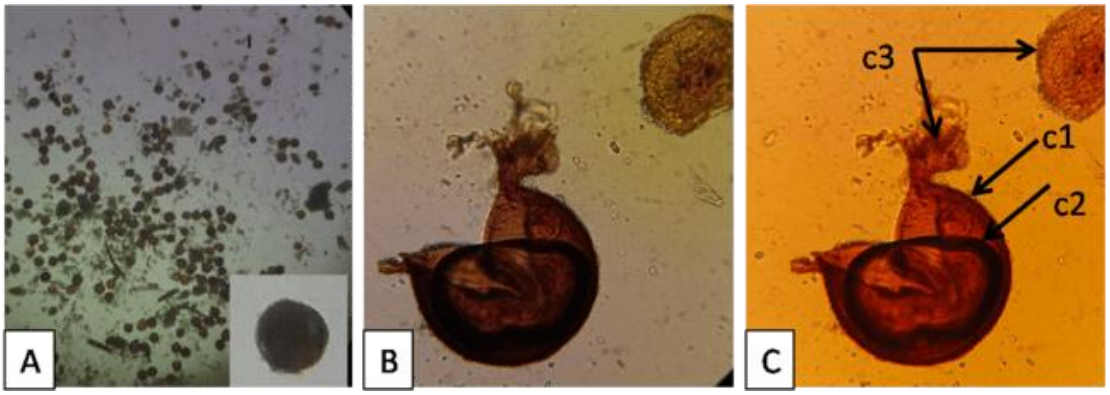

Figure 4. AMF of *Glomus* sp-2. A. Spores of *Glomus* sp-2 on stereo microscope (40 x magnification), B. Spore of *Glomus* sp-2 on PVLG solution (400 x magnification), C. Spore of *Glomus* sp-2 on Melzer solution (400 x magnification) (c1. first layer of spore wall, c2. second layer of spore wall, c3. lipid).

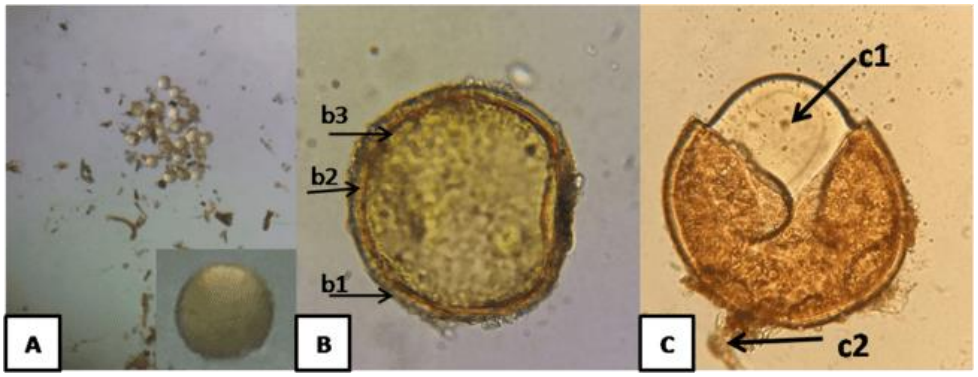

Figure 5. AMF of *Glomus* sp-3. A. Spores of *Glomus* sp-3 on stereo microscope (40 x magnification), B. Spore of *Glomus* sp-3 on PVLG solution (400 x magnification), (b1. first layer of spore wall, b2. second layer of spore wall, b3. third layer of spore wall), C. Spore of *Glomus* sp-3 on Melzer solution (400 x magnification) (c1. lipids, c2. hyphae).

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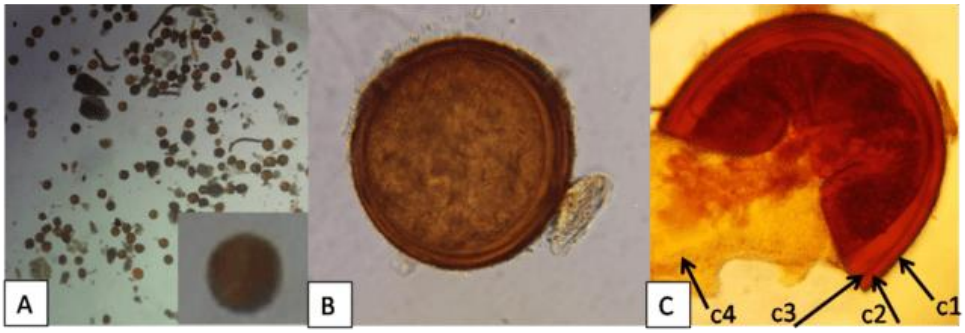

Figure 6. AMF of *Acaulospora* sp. A. Spores of *Acaulospora* sp. on stereo microscope (40 x magnification), B. Spore of *Acaulospora* sp. on PVLG solution (400 x magnification), C. Spore of *Acaulospora* sp. on Melzer solution (400 x magnification) (c1. first layer of spore wall, c2. second layer of spore wall, c3. third layer of spore wall, c4. lipid.).

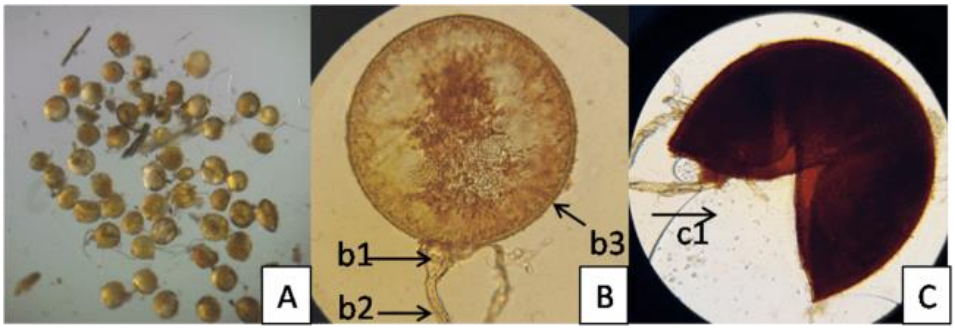

Figure 7. AMF of *Gigaspora* sp. A. Spores of *Gigaspora* sp. on stereo microscope (40 x magnification), B. Spore of *Gigaspora* sp. on PVLG solution (400 x magnification), (b1. bulbous suspensor, b2. hyphae, b3. spore wall), C. Spore of *Gigaspora* sp. on Melzer solution (400 x magnification) c1. lipid.

Figure 8. Discolored (blackened) AMF spores. A. AMF of *Glomus* sp-1. B. AMF of *Glomus* sp-2.

Several spores of *Glomus* sp-1 and *Glomus* sp-2 were found to be black on the inside, which was thought to be caused by parasite infections (Figure 8). AMF spores of glomoid species (such as *Glomus*) are the most susceptible to degradation by parasites such as bacteria and fungi, characterized by changes in the color of the spore interior to

black, orange, red, or brown, and the surface of the spore becomes opaque or appears filmy. Parasitic fungal hyphae can grow and spread in less than 24 hours, even when the spores have been incubated at 4^{o}C. The parasitized AMF spores may also transmit the parasite to other spores (INVAM, 2024). Extraradical spores of AFM are also often susceptible to the parasite infections from various soil microorganisms (Bharathy & Muthukumar, 2023). In addition, it was also stated that parasitized *Glomus dimorphicum* showed symptoms in the form of thick-walled cysts inside of its spores (Boyetchko & Tewari, 1991).

CONCLUSIONS

Based on the results of the study, it is concluded that the highest number of AMF spores in the rhizosphere of peanut plants at Nagari Sawah Tangah, Pariangan Subdistrict, Tanah Datar Regency is from the genus *Glomus* (3 types), namely *Glomus* sp-1 with 15 spores, *Glomus* sp-2 with 12 spores, and *Glomus* sp-3 with 74 spores (a total of 101 spores). The second highest number is from the genus *Acaulospora* (1 type) with 27 spores, and the lowest number of AMF spores is from the genus *Gigaspora* (1 type) with 9 spores per 100 gr of soil sample.

ACKNOWLEDGMENT

The authors would like to thank the Dean of the Faculty of Agriculture who has helped fund this study through the DIPA program of Andalas University in 2019 fiscal year, and all parties who have helped in the implementation of this study.

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