

## EXPLORATION AND MORPHOLOGY IDENTIFICATION OF SPORES ARBUSCULAR MYCORRHIZAL FUNGI FROM HORTICULTURAL PLANTATION

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**Abstract.** Differences in location and rhizosphere cause differences in species diversity and population of Arbuscular Mycorrhizal Fungi (AMF). In addition, not all AMF have the same morphological and physiological characteristics, therefore it is very important to know their identity. This study aims to determine the presence and number of spores as well as to determine the morphological characteristics of AMF originating from the rhizosphere of several horticultural crops in the agricultural land of Payakumbuh State Agricultural Polytechnic. The method used in this research is descriptive exploratory method by means of purposive sampling for soil sampling. While the stages of this research include: taking and collecting data in the field, determining the point of location for soil sampling, taking soil samples, analyzing soil properties in the laboratory, isolating AMF spores and identifying AMF spores morphologically. The conclusions of this study are 1) The population of AMF spores in horticultural land is high. The highest spore population was found in soil samples of the root area of shallot plants (556 spores per 10 g of soil), while the lowest number of spores was in soil samples of eggplant root areas (271 spores per 10 g of soil), 2) AMF exploration in several horticultural crops in the agricultural land of the Payakumbuh State Agricultural Polytechnic, based on morphological identification (shape, color and size), the AMF found consisted of three genera, namely *Glomus* sp, *Gigaspora* sp, and *Scutelospora* sp.

**Keywords:** arbuscular mycorrhizal fungi; horticultural crops; exploration; identification and morphology

### 1. Introduction

Soil as a place to grow plants needs to be preserved because in the soil, especially in the rhizosphere of plants, there are many mikroorganisms that are useful for plants. One of them is the Arbuscular Mycorrhiza Fungi (AMF). Mycorrhiza are known as soil fungi because their habitat in the root area or rhizosphere. Almost 80% of plant species in nature interact or have symbiosis with mycorrhiza. The symbiosis of AMF with plants had been reported 400 million years ago (Selosse *et al.*, 2015). Such types of links are established as a succession of biological processes, which lead to a variety of useful effects in both natural ecosystem and agricultural biotas (Van der Heijden *et al.*, 2015). The diversity and distribution of mycorrhiza varies widely, this can be caused by various environmental conditions. These environmental factors that affect the distribution of AMF are soil structure, P, N nutrients in the soil, organic C content, water, pH, and soil temperature (Hartoyo *et al.*, 2011). Moreover, communal nutrients also relocate from fungi to the plant, along with other related effects, which is probably why AMF improve plant tolerance to biotic and

abiotic factors (Plassard and Dell, 2010). They have the ability to improve characteristics of soil and consequently encourage plant development in normal as well as in stressful circumstances (Navarro *et al.*, 2014). Differences in location and rhizosphere also cause differences in species diversity and AMF population, besides that all AMF do not have the same morphological and physiological characteristics, therefore it is very important to know their identity (Hartoyo *et al.*, 2011).

In Lima Puluh Kota District, especially in the agricultural land area of Payakumbuh State Agricultural Polytechnic, research on mycorrhiza has not been done much, especially regarding its existence (diversity), population and morphological characteristics of AMF spores in the root area or rhizosphere of horticultural crops. Based on reason, it is necessary to explore AMF in the land. One way that can be done to determine the number or population of AMF spores is by means of isolation. Isolation is carried out so that the spores are separated from the soil sample so that the characteristics of AMF spores and their numbers can be known. Meanwhile, to determine the morphological characteristics of AMF spores, morphological identification can be done. Morphological identification was carried out by looking at the shape and color of AMF spores through a microscope. This research aims to determine the presence and number of spores as well as to determine the morphological characteristics of AMF from the rhizosphere of several horticultural crops in the agricultural land of Payakumbuh State Agricultural Polytechnic.

## **2. Methods**

This research was conducted in Biology Laboratory of the Payakumbuh State Agricultural Polytechnic from January until July 2020. The materials used were soil samples from rhizosphere of horticultural crops, glucose solution 60%, PVLG solution, KOH 10%, HCL 2%, dye solution (glycerol, lactic acid, trypan blue), NaOCl 10%, water and distilled water.

The research was conducted with an exploratory descriptive method by purpose sampling for soil sampling (taking soil samples whose location was based on the researcher's consideration). The implementation of research by field survey and laboratory analysis data (soil chemical, morphological identification of AMF spores). The data collected were in the form of qualitative data and species diversity of indigenous AMF. The data were analyzed descriptively by simply arranging and displaying in the form of tables and figures by comparing the data between samples.

### **2.1. Soil sampling.**

Soil samples were taken from the rhizosphere of several horticultural crops (shallots, tomatoes, spring onion, eggplant, cabbage and long beans) located in agricultural land Payakumbuh State Agricultural Polytechnic. At each location,  $\pm 200$  grams of soil samples were taken to observe AMF spores and soil chemical. Soil samples were taken around the plant  $\pm 10$  cm

from the base of the stem or the rhizosphere zone of the plant at a depth of 0-20 cm. Furthermore, the soil samples were dried for 1 week and put in labeled plastic bags to be stored at room temperature (15-20°C) before being analyzed.

## **2.2. Isolation and identification of AMF spores**

### **2.2.1 AMF spore isolation.**

Spore isolation is carried out to separate the spores from the soil sample so that AMF can be identified to determine the spore genus. The method used is the wet sieving. Method from [Dandan and Zhiwei \(2007\)](#). 10 g of soil sample with 3 replications was added with 150 ml of water and stirred for 2 minutes. The suspension was then left to stand for 10 seconds, and the soil fraction was filtered using a stratified sieve measuring 300, 125, and 63 µm. The trapped spores in each filter are taken and filtered on filter paper by spraying water. Then transferred by spraying distilled water to the petridish to observe the type and morphology of spores based on the publication of [Invam \(2014\)](#).

### **2.2.2. Morphological identification of spores**

AMF spores obtained from isolation were identified and counted. Spore identification was carried out by placing the spores on PVLG (Polyvinyl-Lacto-Glycerol) using glass slides. Then attach / close the PVLG with coverslip and press with a rubber pencil tip on each coverslip. Furthermore, it was observed using a microscope with 100x magnification and labeled once identified. The results of the various FMA identification observations were documented in the form of slides.

AMF spores that have been identified to the genus level are collected and counted. Spore counting was carried out directly by transferring the 1 mL spore suspension on a watch glass with four replications. The watch glass is shaken to rotate counter clockwise so that the spores are concentrated in the middle ([Invam, 2014](#)). All spores were observed and their numbers were counted from each genus.

## **3. Results and Discussion**

### **3.1 Soil Sampling Locations**

Soil sampling derived from the rhizosphere of several horticultural crops at the location of the Payakumbuh State Agricultural Polytechnic agricultural land. The altitude at the location of the agricultural land is ± 540 masl. The observation of AMF diversity was carried out at several horticultural planting locations. Soil sampling was based on differences in the types of horticultural plants in the experimental field of Payakumbuh State Agricultural Polytechnic (Purposive Random Sampling). Based on this approach, there are 6 locations for soil sampling on horticultural planting areas. The map of the location for sampling soil samples can be seen in [Figure 1](#).

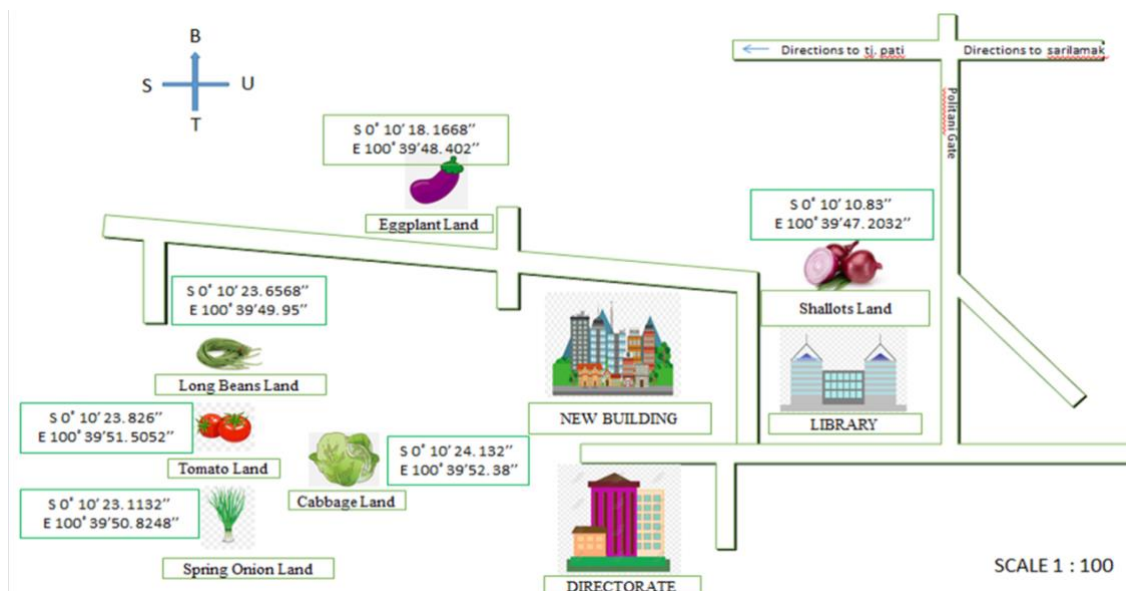


Figure 1. Locations of soil sampling of several horticultural crops in agricultural land Payakumbuh State Agricultural Polytechnic.

### 3.2 The Number of AMF Spores

In Table 1 it can be seen that each soil sample has a different number of spores (per 10 g of soil). Many factors cause differences in the density or number of AMF spores. It caused by differences in soil conditions and content (Meng *et al.* 2021).

Table 1. Spora Density of Arbuscular Mycorrhizal Fungi (per 10 g of soil)

No.	Horticultural plants	Number of spores per sieve size			Amount
		63 $\mu$ m	125 $\mu$ m	300 $\mu$ m	
A.	Shallots	556	0	0	556
B.	Tomato	432	13	0	445
C.	Spring onion	465	26	0	491
D.	Eggplant	268	3	0	271
E.	Cabbage	496	12	0	508
F.	Long beans	409	0	0	409

The density of AMF spores in all soil samples was high. Soil is the most influential factor in the different types and spore density (Mahulette *et al.*, 2021). Soil factors such as soil texture and structure, N and P nutrients, water, and pH significantly affect the distribution of mycorrhizae (Husna *et al.*, 2015). As stated by Puspitasari *et al.*, (2012) that the high spore population caused by environmental conditions that are suitable, optimal and suitable for the growth and development of AMF spores.

Another reason that caused the high spore count was when the research was conducted in the dry season, start from January to July 2019. The dry season or limited water conditions is thought to increase spore sporulation. According to Guaderrama *et al.*, (2014) that the number of spores will vary according to season, AMF colonization is highest during high rainfall conditions, while the number of spores is highest at the end of the dry season.

In [Table 1](#), it can be seen that the lowest number of spores is in eggplant land. The infectivity and effectiveness of mycorrhizae are primarily determined by biotic factors such as the type of mycorrhizae, the host plant, microbial interactions, the type of soil of the host plant, and the composition between fungi and abiotic factors such as environmental factors, especially soil ([Meng et al., 2021](#)).

[Table 1](#) Density of Arbuscular Mycorrhizal Fungi spores (per 10 g of soil). Each type of plant has a different response to AMF types and soil types (related to soil pH and fertility). On the other hand, AMF has differences in the ability to increase nutrient absorption and plant growth, so that it will provide a difference in the level of symbiotic effectiveness between the two (plants and AMF) in the field. It was reported that eggplant plants have a moderate level of dependence ([Samsi et al., 2017](#)) on AMF (Mycorrhizal dependency concept: the relative rate at which plants depend on the presence of mycorrhizal fungi to achieve maximum growth at certain soil fertility levels). If the level of dependence of the plant is high on AMF, then the plant will give a positive growth response to AMF inoculation. This is thought to have caused the low number of AMF spores in eggplant land.

In [Table 1](#), it can be seen that the overall number of spores was found in the 63  $\mu\text{m}$  sieve compared to the number of spores in the 125 and 300  $\mu\text{m}$  sieves. According to [Nusantara et al., \(2012\)](#) that *Glomus* has an average spore size of 50-100  $\mu\text{m}$  so that more spores are found in the 63  $\mu\text{m}$  sieve. It can be concluded that the type most commonly found is *Glomus sp.* The type of *Glomus sp* has a wider distribution than other AMF types. Based on result [Marizal et al. \(2016\)](#) the existence of *Glomus sp* was more dominant than other types of AMF indigenous. Various studies have reported that *Glomus* species are dominant in various ecosystems. As reported by [Maria \(2007\)](#), *Glomus* is the dominant species found in the three land use types in Sumberdaya Lampung, followed by *Gigaspora*, *Acaulospora* and *Scutelospora*. Likewise, reported by [Susila \(2017\)](#) that the dominant *Glomus sp* species was found in the shallots rhizosphere from three growing locations with different plains (low, medium and highlands). *Glomus sp* was also found in all sample locations of citronella plants in West Sumatra (Balai Batu, Laiang and Simawang) ([Armansyah et al., 2018](#)).

### 3.3 Soil Analysis Observations

One of the factors that determine the growth and density of AMF spores is the condition and chemical content of the soil. The results of soil analysis on the pH of  $\text{H}_2\text{O}$  from the six samples ranged from 5.5 to 6.6. The available P and C-organic values from the six locations were classified as very high ([Pusat Penelitian dan Pengembangan Tanah, 2012](#)).

Spore germination can be influenced by environmental factors such as pH, temperature, soil moisture and organic matter. Based on the chemical soil pH of the six soil samples was

classified as acidic to slightly acidic. Soil pH can affect the activity of enzymes that play a role in germination, growth and plant development. AMF will be effective when applied to less favorable soil. According to [Danesh \*et al.\*, \(2007\)](#), AMF has the specific ability of these species to increase plant growth in unfavorable soil conditions by forming extensive hyphae in the soil (innate effectiveness) to absorb nutrients and water.

**Table 2.** Results of Analysis of Chemical Soil Properties in Rhizosphere of Several Horticulture Crops in Agricultural Land Payakumbuh State Agricultural Polytechnic.

No.	Plant Sources	pH H <sub>2</sub> O	P Available Bray II (ppm)	C Organic (%)	Water Content (%)
1.	Shallots	6.00	24.20	5.50	10.26
2.	Tomato	5.50	23.10	8.47	16.40
3.	Spring onion	5.50	198.00	8.14	16.50
4.	Eggplant	6.50	31.90	7.92	14.40
5.	Cabbage	6.50	275.00	6.60	13.14
6.	Long beans	5.50	34.10	7.48	18.20

Source: *Land Laboratory of Payakumbuh State Agricultural Polytechnic (2020)*

The land where the soil sampling was carried out was the Payakumbuh State Agricultural Polytechnic experimental land. The land is often fertilized with organic matter (manure) and chemical fertilizers (SP<sub>36</sub>). Fungi participate in decomposition of organic matter and deliver nutrients for plant growth. Their role is very important in plant protection against pathogenic microorganisms as biological agents, which influences soil health ([Frąc \*et al.\*, 2015](#)). AMF significantly affects the concentrations of P and N and the N:P ratio in plant ([Wang \*et al.\*, 2012](#)). This is reinforced by the results of [Susila \(2018\)](#) study which reported that the availability of P was very strong with root colonization, where the higher the P the lower the colonization that occurred between AMF and plant roots. If external hyphal growth is inhibited.

According to [Ishii \(2004\)](#), P concentrations in the medium that exceed 50 ppm will inhibit AMF colonization. However, the optimal amount of P depends on the plant and the type of AMF. Susila reported that the number of spores increased as the P available. At available P 63.45 ppm, there was a decrease in the number (population) of AMF. It can be seen in [Table 2](#) that although the available P is much higher than other sample plants (exceeding 50 ppm), the number of spores per gram of soil is still relatively high.

The availability of C-organic is related to soil fertility. If the soil has low availability of C-organic (<1), the soil fertility is low, characterized by a lack of macro and micro nutrients because one of the functions of organic matter is to provide macro and micro nutrients ([Pusat Penelitian dan Pengembangan Tanah, 2012](#)). In fertile soil the roots are actively working while the symbiosis with AMF decreases. This is related to the principle of mutualistic symbiosis where when the soil fertility is good, plants will not ask for help in other microorganisms, including AMF.

[Table 2](#) shows that the C-organic in all sample soils is very high (> 5) ([Pusat Penelitian](#)



dan Pengembangan Tanah, 2012). However, in soil that has a pH below neutral, the availability of other macro and micro nutrients can decrease. This can cause the spores to be still effective so that overall, the availability of spores is still relatively high per gram of soil. Table 2 shows that the higher the soil organic C content, the number of spores decreases. This is because the more organic matter in the soil, it will affect soil moisture which causes low AMF sporulation. One of the roles of organic matter is to increase the soil's ability to hold water. According to the opinion of Hardjowigono (2010), the amount of organic matter will affect the soil moisture status. In moist soil conditions, the sporulation process (spore formation) of AMF becomes lower so that the number of spores contained in the soil is also small (Burhanuddin, 2012). In the sample soil of shallots, with the lowest C-organic content among the other sample soils, the number of spores was the highest.

Water content affects the number of AMF spores. Various studies have reported that the number of AMF spores is more found in soils that have a low water content than those with a high moisture content. During the dry season with limited water availability, AMF will form spores to survive, so that the number of spores will increase (Guaderrama *et al.*, 2014). If the soil water content contains enough water, it will stimulate the spores to germinate so that the number of spores decreases. Elita *et al.*, (2018) reported that the application of AMF to rice cultivation using the SRI method (System of Rice Intensification Method) showed a high level of root colonization and spore count. In Table 2, it can be seen that the highest number of spores on shallot land with the lowest water content is 10.26%.

### 3.4 Morphological Identification of AMF Spores

The identification of arbuscular mycorrhizal fungi is carried out based on the similarity of spore morphological characteristics including color, spore shape, size and ornament Invam (2014). The stages of AMF identification are as follows:

1. Color of spores: using the standard color chart that is commonly used. The colors of mycorrhizal spores range from hyaline yellow, greenish yellow, brown, reddish brown to blackish brown.
2. Spore shape: in general, the shape of the spores is globe, sub globose, oval.
3. Spore size,
4. Has ornament and spore contents such as bulbous, spines, fine threads such as hair, spines, and others.

Identification of AMF which has abundant spores and is found in the rhizosphere of all observed horticultural plants is as follows *Glomus* sp. The spores found were round to oval in shape, the color of the spores ranged from light yellow, brown to reddish brown and shiny black. Spore walls are slippery. Pass the 125  $\mu\text{m}$  and 250  $\mu\text{m}$  sieve (Figure 2).

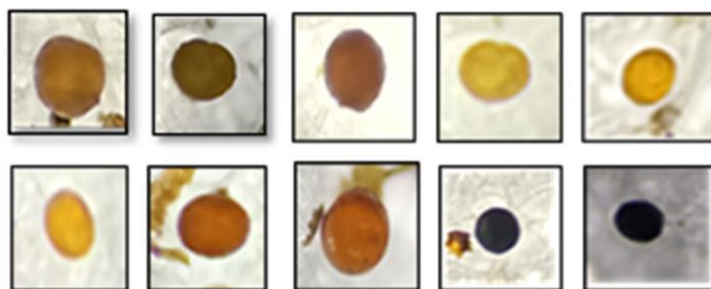


Figure 2. Different Types of *Glomus sp* Derived from Several Rhizosphere Horticultural Crops

The genus *Glomus* is characterized by a round to oval shape, the spore wall consists of more than one layer, smooth. The color of the spores of the genus *Glomus* varies from yellow, brownish yellow, reddish brown, clear white (hyaline), light brown, to dark brown and blackish (Invam, 2014). There is often a substanding hypha, size 108- 341  $\mu\text{m}$ .

*Gigaspora* has a bulbous suspensor. Spores are round and slightly rounded. The color of the spores is yellow, reddish brown to blackish. Has a smooth, single-layered spore wall, has no ornament. The spores are relatively large, passing through a 250  $\mu\text{m}$  filter (Figure 3).

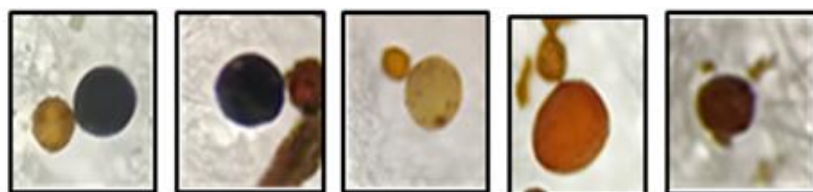


Figure 3. Different Types of *Gigaspora sp* Derived from Several Rhizosphere Horticultural Crops

The genus *Gigaspora* has features; single spores in the soil (143-500  $\mu\text{m}$ ), form globose or sub globose; yellow, brownish yellow, to blackish, slimy white, golden yellow (Schenck and Perez, 1990), the contents of the spore contain an oil-like fluid, do not have ornamentation, the hyphae form bulbous suspensors or rounded hyphae holders, spores grow at the tip of the "bulbous suspensor" (hyphae bulging like tiny spores) germ tube emerges directly from the spore wall (Invam, 2014).

*Scutellospora sp.* The shape of the spores is round to irregular, the color of the spores is clear, dirty, gray to black, the surface of the spores is smooth, the walls are rather thick. There is a germination shield. In spores rather dirty. Pass the 125  $\mu\text{m}$  sieve (Figure 4).

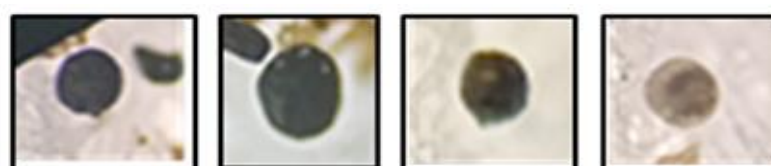


Figure 4. Different Types of *Scutellospora sp.* Derived from Several Rhizosphere Horticultural Crops



The genus *Scutelospora* has features; produce single spores in the soil, in the form of globos or sub globos and sometimes irregular; measuring 58 - 500  $\mu\text{m}$ , hyaline, brownish yellow, red, have two layers of thin spore walls, have a germination shield (Invam, 2014).

#### 4. Conclusions

The conclusions of this study are 1) The population of AMF spores in horticultural agricultural land is high. The highest spore population was found in soil samples of the root area of shallot plants (556 spores per 10 g of soil), while the lowest number of spores was in soil samples of eggplant root areas (271 spores per 10 g of soil), 2) AMF exploration in several horticultural crops in the agricultural land of the Payakumbuh State Agricultural Polytechnic, based on morphological identification (shape, color, size, and ornament), the AMF found consists of three genera, namely *Glomus sp*, *Gigaspora sp* and *Scutelospora sp*.

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