Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 3 No. 2 October 2024

Characterization of Indigenous Arbuscular Mycorrhizal Fungi in Seedlings and Production of Pepper (Piper nigrum L.) Cultivation

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ABSTRACT. Mycorrhizal plants are known to be only slightly attacked by diseases caused by fungi. This condition can be used as an alternative to control biological diseases in pepper plants. The study was conducted to explore the diversity of arbuscular mycorrhizal fungi in pepper plants in nursery and production areas, which have the potential to be used as biological agents in controlling stem rot disease caused by Phytophthora sp. fungi. Samples were taken from three fields in Kedarpan Village, Kejobong District, Purbalingga Regency. The study was a survey experiment that took samples intentionally (purposive sampling). ata on the intensity of root infection to determine its diversity were analyzed using Analysis of Variant. Data from several observation variables will be analyzed descriptively, qualitatively, and quantitatively. Morphospecies identification was carried out on spores of Arbuscular Mycorrhizal Fungi that were successfully isolated. Arbuscular Mycorrhizal Fungi that were successfully isolated and identified from pepper plantations consisted of 5 genera of fungi, with morphospecies diversity in each genus. In the pepper nursery land, there were the genera Glomus, Gigaspora, Acaulospora, and Entropospora; the pepper production land of the Margo Utomo Farmers Group had the genera Glomus, Gigaspora, Scutellospora, and Entropospora; while the pepper production land owned by residents had Glomus, Gigaspora, and Entropospora. Glomus was found at all sampling locations. The level of infection or colonization of AMF in pepper plantations in the three locations observed showed a high level of infection (>30%). However, in each pepper plantation area, AMF did not significantly affect the intensity of root infection ($F = 6.114$; $p = 0.003$).

Keywords : *Arbuscular Mycorrhizal Fungi, nursery land, production land, pepper rhizosphere*

INTRODUCTION

Pepper (Piper nigrum L.) is one of the spices used in cooking. Pepper can also be used as an oil ingredient, as herbal medicines, as a mixture of making, drinks, to prevent the development of breast cancer (extracted with turmeric), help to treat nausea, loss of appetite, curing gout, ; pepper is also used as a cosmetic production and as a balm in the form of cream (Sulhatun et al., 2013). The main contents include piperine alkaloids (5.3-9.2%), kavicine (up to 1%), methyl-pyrroline, starch (36-37%), and crude fiber (±14%). (Hikmawanti et al., 2016).

Indonesia ranks second in the world with an average export value in 2004-2028, reaching US$ 232,266. This pepper commodity can also be seen controlling a relatively large export market share, which shows competitiveness in the international market (Balqis & Yanuar, 2021).

Purbalingga Regency is one of the pepper development areas in Central Java province, in the last five years it has become a pepper production center in Java, with an area of 583.94 hectares. In 2016, it produced 182 tons of pepper. Pepper farming is developing in 7 sub-districts, 2 of which are production centers, Kejobong Sub-district, and Pengadegan

Sub-district. Pepper farming productivity in this area reaches 311 kg/ha, lower than national pepper productivity, which averages 572 kg/ha compared to pepper productivity in Vietnam, which reaches 2.5-3.2 tons/ha (Ardana et al., 2017).

The decline in production is caused by several factors, including the most feared by pepper farmers, namely climate change and OPT attacks. Climate change significantly affects the development of diseases, very high rainfall causes flooding around pepper plantations. This condition makes pepper plants susceptible to disease, a very feared disease, namely, Basal Stem Rot (BPB), the spread of this disease is swift and deadly quickly (Suhaendah et al., 2016). One of the efforts implemented to improve quality standards and increase soil fertility requires alternative use of environmentally friendly technology.

Arbuscular Mycorrhizal Fungi (AMF) help increase the efficiency of nutrient absorption, can be used as biological fertilizers and biological agents, and fertilize the soil. (Sukmawati et al., 2021). AMF is a fertilizer that is only sufficient to be given once in the plant's life as long as there is a host plant, because it is a living thing that can continue to grow (Setiadi & Setiawan, 2011).

Although the role of AMF is known, studies on the characterization and identification of AMF from pepper plantations are rarely carried out. According to Ramadhani et al., (2019), almost 70% of AMF research is directed at plant growth benefits and less than 15% of studies characterization and identification in plantations. Therefore, this study was conducted to determine the characterization and identification of AMF in pepper plantations in Kedarpan Village, Kejobong District, Purbalingga Regency, which have the potential to be used as biological agents in controlling stem rot disease caused by Phytophthora sp. The problem studied in this study is how to characterize Arbuscular Mycorrhizal Fungi (AMF) in the rhizosphere of pepper plants (Piper nigrum L.) in nursery and pepper cultivation production areas in Kedarpan Village, Kejobong District, Purbalingga Regency.

MATERIALS AND METHODS

The research was conducted in the experimental garden of the Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto, Karangsari Village, Kembaran District, Banyumas Regency, Basic Agrotechnology Laboratory and Applied Agrotechnology Laboratory of the Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto, implementation from August 2022 to May 2023.

The tools used include pots, soil trowels, plastic, markers, analytical scales, label paper, and soil sterilizers. Equipment for laboratory observations, namely: beaker glass, measuring cup, preparation, cover slip, scissors, gauze, mattress thread, glass object, spatula, dropper pipette, petri dish, small cup glass, tweezers, hot plate, spray bottle, basin, centrifuge, funnel, stereo microscope, light microscope, laptop, camera, and stationery. The materials used include soil samples from nursery and production land, water, corn seeds, zeolite, latex gloves, filter paper, distilled water, lactic acid, glycerol, 60% sucrose solution, commercial vinegar, KOH, Parker Quik Ink. The research is a survey experiment utilizing purposive sampling, ; the data obtained are described qualitatively and quantitatively.

Table 1. Soil Sampling in Kedarpan Village, Kejobong District, Purbalingga Regency

Village	Sampling			
	Land 1	Land 2	Land 3	Number of Samples
Kedarpan	65 points	57 points	13 points	
Number of samples in composite				135 samples

Sample description:
L1: nursery land
L2: KTMU production land, L3: Residents' production land

Sampling is based on homogeneity, namely the area of land spread, plant age, and differences in each land. The first step to determine the location for sampling, which is in Kedarpan Village, Kejobong District, Purbalingga Regency. In Kedarpan Village, there are three lands nursery land, Margo Utomo Farmers Group (KTMU) production land, and production land owned by residents. Each land is taken 2-3 soil samples weighing ±100 grams. Samples are taken in the rhizosphere area of pepper roots; samples are taken from the nursery land of as many as 65 samples, the production land of the Margo Utomo Farmers Group of as many as 57 samples, and production land owned by residents of 13 samples. Sampling using plastic bags, samples are composited so that the total is 135 samples.

Determination of Sample Points

Soil sampling was carried out on three pepper plantations with different lands: the farmer group nursery land, the farmer group production land, and the production land owned by Kedarpan Village, Kejobong District, Purbalingga Regency resident. Determination of soil sample points was carried out diagonally to obtain five sample points (Kafrawi et al., 2022).

Soil Sampling

Soil sampling was carried out by removing the grass around the pepper plants, then digging the soil about 50 cm until the roots of the pepper plants were visible, taking the soil near the roots of the pepper plants, after which it was put into a plastic bag that had been coded.

Preparation of Planting Media for Trapping Culture

The planting medium used is zeolite. Zeolite sterilization is done by heating at a 80 0C for 8 hours (Anisa & Susan, 2012). Sterile zeolite is put into 18 cm x 12 cm pots containing 1.5 kg as many as 135 pots, ; the planting medium is watered using a watering can until it drips. A 100 g soil sample is inserted into the planting medium by making a hole in the planting medium, and the soil sample is placed in the hole. The pot is coded according to the code on the sample.

The corn seeds used are the Bonanza Now F1 variety. Corn seeds can be planted by placing selected seeds in the plating hole treated with pepper rhizosphere soil samples. One hole is planted with one corn seed, the planting hole is covered with soil and zeolite.

Maintenance

Maintenance includes watering, weeding, and fertilization. Fertilization is carried out once every 15 days after planting. The fertilizers given are Urea with a dose of 0.4 g/pot, SP36 with 0.3 g/pot, and KCL with 0.4 g/pot. The fertilization method is envolves making three holes 2 cm deep, inserting the three fertilizers into each hole, and then closing each hole again.

Spore Harvesting

Spore harvesting is carried out after the host is 60 days old after planting by cutting the base of the stem plant using a knife, the planting medium is left for two weeks (stressing), and the aim is to stimulate spore growth. After being gone for 2 weeks, the spores and zeolite media are put into plastic for observation in the laboratory (Sukiman, 2021).

Spore Count Calculation

Spore count calculation using the spore filtration method. First, a sample of corn rhizosphere soil is prepared, a 25 g soil sample is weighed, the soil is poured into a beaker, and 1000 mL of water is added to the beaker and stirred using a spatula. The solution is poured into a graduated sieve into various diameter sizes (425 µm), (212 µm), (106 µm), and (63 µm). Add water until it is not cloudy, and rinsed using running water.

The results of the filtration of diameters (425µm), (212µm), (106 µm), and (63µm) were poured into a beaker. Then it is put into a centrifuge tube wiyh the addition of sugar solution (sucrose solution 60%), twice the volume of the extract, and centrifugated at a speed of 3000 rpm for 5 minutes. The centrifugation results consist of 3 layers, water, sugar solution, and sediment. The filtration results were poured onto the surface of the filter paper with the help of a funnel and immediately washed with running water so that the spores did not dissolve. The spores were transferred to a petri dish lined with filter paper and observed under a stereo microscope (Dharmaputri et al., 2016).

Calculation of Root Infection Intensity (%) AMF

The steps for observing the intensity of infection are washing the roots until clean, soaking them in 10% KOH, and heating them at a temperature of 90 0C for 9 minutes. The roots were rinsed with running water, using a sieve soaked in lactoglyserol, 5 ml Parker Quik ink, 5 % vinegar (2:2:1), and heated at 90 0C for 3 minutes. The roots were soaked in a mixture of 5% vinegar and water in a ratio of 1:1 to reduce excess dye solution from Parker Quik ink. 1 cm long roots were placed in a row on a glass object. Every five pieces of root were covered with a cover slip. Observe each piece of root under a light microscope and calculate the intensity of root infection. The intensity of root infection was measured by looking at the percentage of roots infected by AMF. Measurement of the intensity of root infection by AMF was carried out using a staining technique (Setyaningsih et al., 2020). The percentage of infected roots was calculated using the following formula:

$$\% \text{ Infection Intensity} = \frac{\sum infected\ root\ cuttings}{\sum number\ of\ root\ examples} \text{ x } 100\ \%$$

Table 2. Category of Root Infection Intensity by AMF

Percentage of infection	Category
0	Not infected
< 10	Low
10-30	Medium
> 30	High

Source : (Connor *et al.*, 2015).

Spore Identification

The preparation of spore preparations is intended to assist in the identification process from these preparations, it is expected that spore morphological information can determine the genus of AMF. Identification is carried out using a compound microscope with the help of a binocular microscope and spore tweezers. The spores obtained are collected based on the morphological characteristics of mycorrhizal spores, including: spore shape, spore size, spore color, hyphae attachment, and spore ornament.

Identification and classification of Arbuscular Mycorrhizal Fungi (AMF) are adjusted according to the International Culture Collection of Vesicular Arbuscular Mycorrhizal (INVAM). Spores can be identified based on the size and color of the spores, the spore wall layer, the reaction with Melzer's solution (lipid droplet), spore ornament (outer wall of the

Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 3 No. 2 October 2024

spore), and the shape of the hyphae attached to the spore wall (bulbous suspensor and subtending hyphae, or hyphae seat) (INVAM, 2017).

Identifying the color, number, and thickness of spore walls, and the presence or absence of typical structures and shapes of Arbuscular Mycorrhizal Fungi spores based on spore observations under a microscope, the microscopic characteristics of the spores found are then matched with the identification guidelines (INVAM, 2017), to determine the genus and morphospecies of Arbuscular Mycorrhizal Fungi found.

The root infection intensity data obtained will be normalized, homogenized, and then analyzed to determine its diversity using Analysis of Variants. Data from several observation variables will be analyzed descriptively, qualitatively, and quantitatively.

RESULTS AND DISCUSSION

Results of the Average AMF Spore Population

The results of the AMF spore inventory in Kedarpan Village on three different lands showed that land 1 in the nursery had the highest number of spore populations in the genus Glomus. Land 2 in the Margo Utomo Farmers Group production land showed the highest spore populations in the genus Glomus. Land 3 in the production land owned by residents showed the number of genus Glomus and genus Gigaspora, and the number of spore populations was equivalent.

Table 3. Number of Identifiable Spores from Each Genus Obtained in Kedarpan Village in 3 Research Fields

Village	Lands	The number of spores from the genus obtained from 10 g of sample				
		Glomus	*Gigaspora*	*Acaulospora*	*Scutellospora*	*Entrospora*
Kedarpan	L1	32	3	5	-	1
	L2	7	5	-	1	1
	L3	3	3	-	-	1

Note: The location code refers to Table 3.1. (-) means no AMF spores were found. (10 g) of sample is the same as the spores in the zeolite propagation medium

Arbuscular Mycorrhizal Fungi on Pepper Plant Rhizosphere in Kedarpan Village

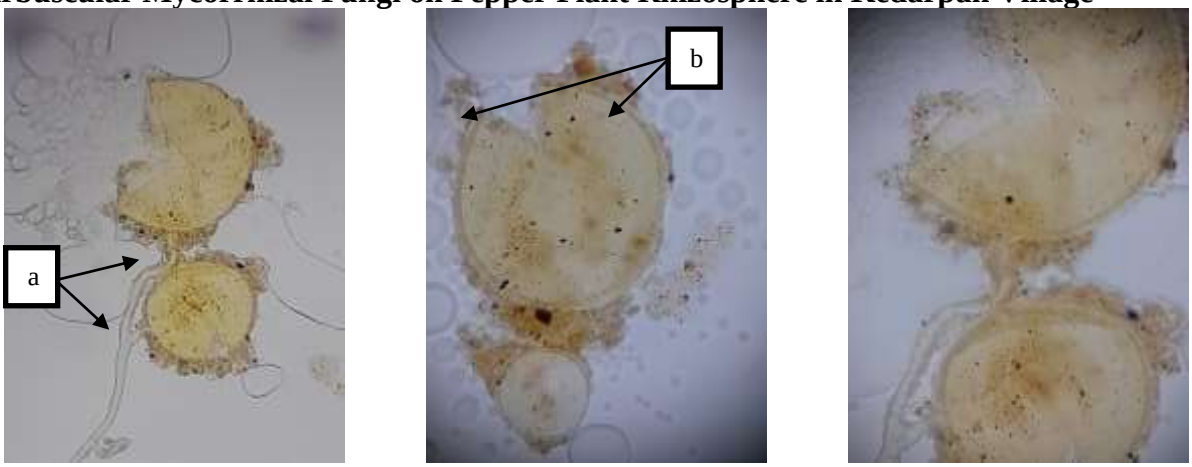

Figure 1. Arbuscular Mycorrhizal Fungi Spores Genus Glomus in Pepper Nursery Land in Kedarpan Village, Kejobong District, Purbalingga Regency. Description: Glomus sp. Spores of the Glomus genus are yellowish, round in shape with a diameter of 100-120µm, forming the characteristic of Glomus on (a) subtending hypha, (b) spore Wall.

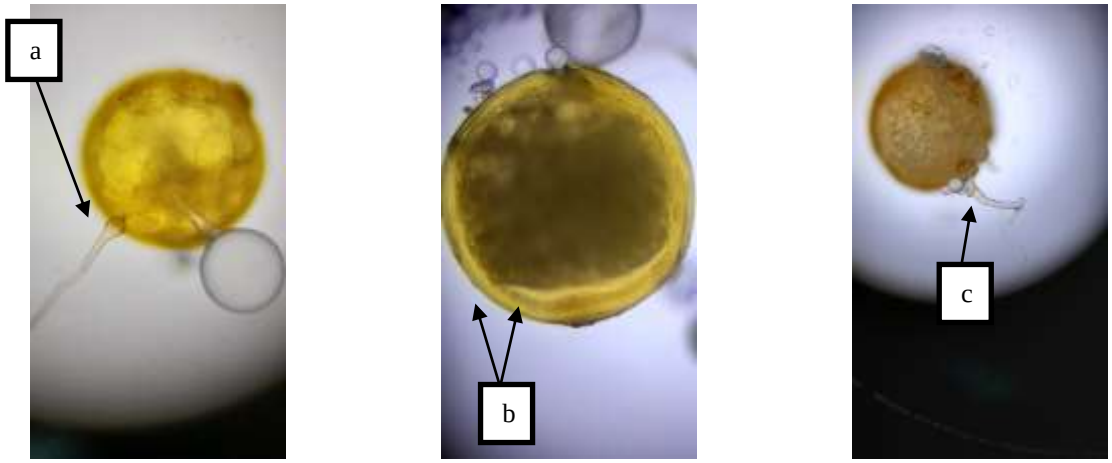

Figure 2. Spores of Arbuscular Mycorrhizal Fungi Genus Gigaspora in Pepper Nursery Land, Kedarpan Village, Kejobong District, Purbalingga Regency. Description: Gigaspora sp. Gigaspora spores are yellow in color with large to substantial sizes, 270-420µm in diameter, containing (a) bulbous suspensor, (b) spore Wall, with (c) bright apparent hyphae.

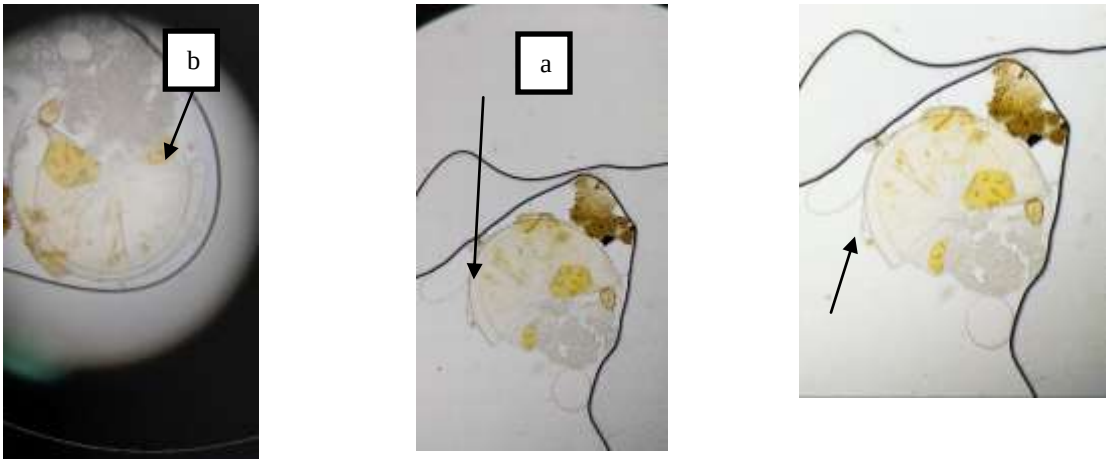

Figure 3. Spores of Arbuscular Mycorrhizal Fungi Genus Scutellospora in Pepper Production Land of Margo Utomo Farmer Group in Kedarpan Village, Kejobong District, Purbalingga Regency. Description: Scutellospora sp. Genus Scutellospora with clear spores, 380µm in diameter, containing (a) germination- shield and (b) bulbous suspensor, (c) spore Wall.

Arbuscular Mycorrhizal Fungi belong to the endomycorrhizal group characterized by intracellular and extracellular hyphae that penetrate the cortex from one cell to another. Inside the cell are twisted hyphae or branched hyphal structures called arbuscules. The presence of arbuscules plays a role in facilitating the process of identifying plants, and whether or not an infection has occurred in the plant's roots (Bonfante, 2014). Furthermore, it is said that all arbuscular mycorrhizal fungi, including the genera Gigasporae, Scutellospora, Glomus, Entropospora, and Aacaulosporae, can form arbuscules. The main characteristic of AMF is the presence of arbuscules in the root cortex. Initially, the fungus grows between the cortex cells, then penetrates the host cell wall and develops inside the cell (Read, 2014).

Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 3 No. 2 October 2024

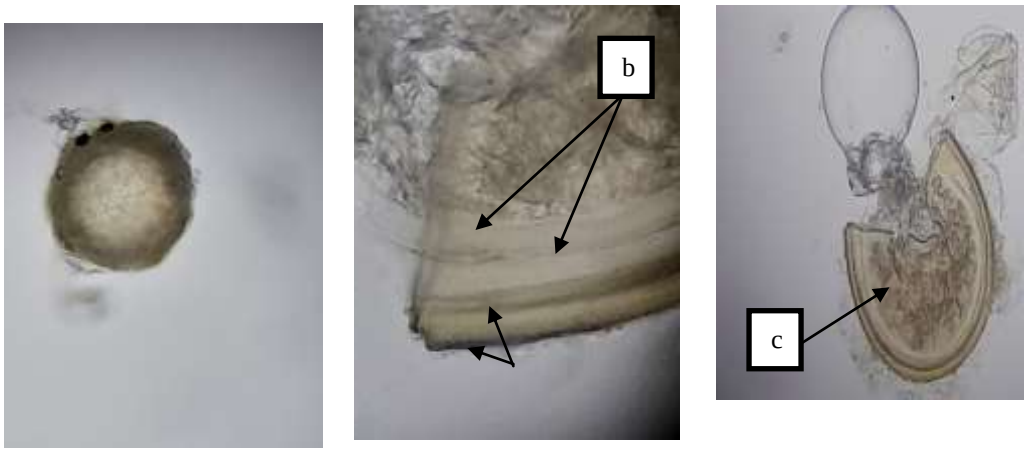

Figure 4. Spores of Arbuscular Mycorrhizal Fungi Genus Acaulospora in Pepper Nursery Land, Kedarpan Village, Kejobong District, Purbalingga Regency. Description: Acaulospora sp. Acaulospora spores are transparent, and round with a 140-150 µm diameter. Spores form 2 walls, namely (a) spore-wall, and (b) germinal-wall. On the surface of the spore- wall, (c) distinctive ornaments characterize the species.

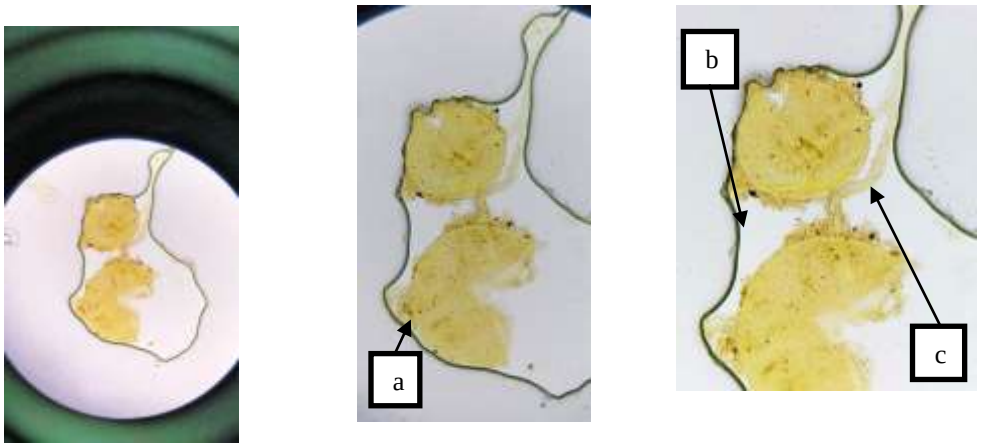

Figure 5. Spores of Arbuscular Mycorrhizal Fungi, lab code D-130, Genus Entrospora on Pepper Production Land Owned by Residents of Kedarpan Village, Kejobong District, Purbalingga Regency. Description: Entrospora sp. Spores of the genus Entrospora are round, yellowish in color, with a diameter of 100 µm. There are two walls (a) (spore-wall and germinal-wall), and there are no ornaments on the surface of the spore-wall. There is a (b) saccule with a position opposite to (c) subtending hyphae.

AMF Infection in Pepper Nursery and Production Lands

The presence of internal hyphae, external hyphae, vesicles, and arbuscules indicates infection of plant roots by AMF. Vesicles are fungal structures that originate from swelling of internal hyphae and are oval or round in shape, containing fatty fluid, which functions as a food storage organ or develops into chlamydospores, which function as reproductive organs and resistant structures. Vesicles usually form more outside the cortex tissue in old infection areas after forming arbuscules. If the metabolic supply from the host plant decreases, the fungus will use the food reserves so that the vesicles degenerate (Mahfut et al., 2020).

Table 5. Density analysis and identification of AMF spores to the morphospecies level, pepper nursery land, Kedarpan Village, Kejobong District, Purbalingga Regency

No.	Sample Code	Genus	Number of spores per 10 g	Description
1.	L1 (1)	1 *Glomus*	106	Spores of the genus Glomus are clear to yellowish in color, round in shape with a diameter of 70-80 µm. Spores consist of only one wall (spore wall).
2.	L1 (2)	2 *Glomus*	358	Spores of the genus Glomus are yellowish; the diameter of the spores is 130-140pm. Spores only form a spore wall, forming spores in the roots.
3.	L1 (3)	3 *Glomus*	4	Yellowish spores from Glomus have a diameter of 100pm and only form spore walls.
4.	L1 (4)	4. *Glomus*	219	Spores of the Glomus genus are yellowish in color, oval to oblong in shape, with a diameter of 90-130x150 µm, only forming spore walls, and the characteristic feature in the subtending hypha is that the spore wall layer is visible.
5.	L1 (8)	5. *Acaulospora*	175	Spores of the genus Glomus that are yellowish, oval to oblong in shape, with a diameter of 90-130x150µm only form spore walls, and the characteristic in the subtending hypha is that the spore wall layer is visible.
6.	L1 (9)	6. *Glomus*	72	Spores of the genus Glomus that are yellowish and round in shape, with a diameter of 90-100µm, only form one wall, namely the spore wall.
7.	L1 (14)	7. *Glomus*	6	The genus Glomus's spores are yellow and round, with a diameter of 130-140µm; there are only spore walls.
8.	L1 (18)	8. *Acaulospora*	12	Spores of the genus Acaulospora are clear to yellowish in color, with a diameter of 100-120µm, and have ornaments on the spore wall, which are characteristic of this genus and determine its species.
9.	L1 (19)	9. *Glomus*	15	Glomus spores are yellowish round with a diameter of 90-100µm and only form one wall, namely the spore wall, without ornamentation.
10.	L1 (20)	10. *Glomus*	7	The spores of the Glomus genus are yellowish in color and round in shape with a diameter of 100-120µm, forming the characteristic Glomus characteristic of the subtending hypha.
11.	L1 (23)	11. *Glomus*	83	Spores of the genus Glomus formed in the roots are oval with a diameter of 60x80-90µm; spores only form spore walls.
12.	L1 (24)	12. *Glomus*	76	Genus Glomus with a yellow color, spores are round and oval in shape and have a diameter of 120-150µm. Spores only form spore walls.
13.	L1 (28)	13. *Glomus*	563	Spores of the genus Glomus are yellow, round, and oval in shape with a diameter of 70-130µm. Spores only form spore walls.
14.	L1 (32)	14. *Glomus*	13	Spores of the genus Glomus are yellow round with a diameter of 70-100µm. There are only spore walls.
15.	L1 (33)	15. *Glomus*	2	Spores of the genus Glomus are yellowish to yellow spores and are round with a diameter of 100-110µm. Spores only form spore walls.
16.	L1 (36)	16. *Entrospora*	1	Entrospora's spores are transparent and round, with a diameter of 80x80µm. The spores have a distinctive feature, namely forming a saccule, and the position of the subending hypha is opposite. There are no ornaments on the spore wall.
		17. *Glomus*	1	Glomus spores are yellowish with a diameter of 70-90µm. The spores only have a spore wall.

Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 3 No. 2 October 2024

17.	L1 (38)	18. *Acaulospora*	14	Acaulospora spores are transparent and round with a 140-150µm diameter. Spores form 2 walls, namely the spore wall and the germinal wall. On the surface of the spore wall, distinctive ornaments characterize the species.
18.	L1 (39)	19. *Glomus*	456	Glomus spores are formed in the roots—tiny, round spores, yellowish in color, with a diameter of 130x130µm.
19.	L1 (88)	20. *Glomus*	272	Glomus spores are formed inside the roots. Spores are yellowish in color with a diameter of 70-130µm for the round ones. In comparison, the oval ones have a diameter of 50-80x100-140µm.
20.	L1 (1)2	29. *Glomus*	736	The spores of the Glomus genus are yellowish in color with a diameter of 110-130 µm, with distinctive characteristics in the spore-wall layer and hyphae attachment.
21.	L1 (2)2	30. *Glomus*	7	Spores of the genus Glomus are clear to yellowish in color, and round in shape with a diameter of 110-150µm. Spores are characterized by only forming a spore wall.
22.	L1 (3)2	31. *Gigaspora*	6	Gigaspora spores are yellow with a large to enormous size, a diameter of 270-420µm; there is a bulbous suspensor with bright apparent hyphae.
23.	L1 (5)2	32. *Glomus*	129	Spores of the genus Glomus are yellowish, round to oval in shape with a small to medium size, namely a diameter of 40-130µm; spores are composed of spore-wall only.
24.	L1 (6)2	33. *Glomus*	92	Spores of the genus Glomus are yellow and round in shape with a diameter of 120-130µm; the spores are composed of spore walls only.
25.	L1 (7)2	34. *Glomus*	36	Spores of the genus Glomus are yellowish with a diameter of 100-110µm; spores are composed of only one wall.
26.	L1 (9)2	35. *Glomus*	136	The genus Glomus's spores are yellow and round in shape with a diameter of 110-150µm, spores are composed of only 1 type of wall.
27.	L1 (10)2	36.*Gigaspora*	358	Spores of the genus Gigaspora are clear, small, and round in shape with a diameter of 140µm; there are other characteristics in the form of bulbous suspensors.
28.	L1 (12)2	37. *Glomus*	326	Spores of the genus Glomus are yellowish round in shape with small to medium size, diameter 30-120µm.
29.	L1 (13)2	38. *Glomus*	10	Spores of the genus Glomus are yellow, medium-sized, and round in shape. The diameter of the spores is 120-140 µm. Spores are composed of only spore walls.
30.	L1 (14)2	39. *Acaulospora*	539	Spores of the genus Acaulospora are clear in color with a round shape measuring 190µm in diameter; there are saccules and ornaments on the spore walls.
		40. *Glomus*	214	Spores of the genus Glomus are yellow, with a diameter of 130µm; the spores only have spore walls.
31.	L1 (16)2	41. *Glomus*	175	Spores of the genus Glomus are yellowish, round to oval in shape, diameter 50-70x100-170µ, spores are composed of 1 spore-wall, and there are typical Glomus characteristics on the subtending hyphae.
32.	L1 (19)2	42. *Glomus*	19	Spores of the genus Glomus are yellowish round in shape with a diameter of 110-130µm; spores are composed of 1 type of wall.
33.	L1 (21)2	43. *Glomus*	38	Spores of the genus Glomus that develop in the roots are round, with a diameter of 80-120µm.
34.	L1 (25)2	44. *Glomus*	692	Spores of the genus Glomus are clear to yellowish in color, round in shape, 60-100µm in diameter, and consist of only one wall.

35.	L1 (31)2	45. *Glomus*	267	Spores of the genus Glomus are clear to yellowish, round in shape, 60-100µm in diameter; spores consist of only one wall.
36.	L1 (37)2	46. *Glomus*	31	Spores of the genus Glomus are clear to yellowish, oval, 40-100x50-140µm in diameter; spores develop in the roots.
37.	L1 (38)2	*47. Glomus*	66	Spores of the genus Glomus are yellow, measuring 110-120 µm in diameter; in their spores, only spore walls are formed.
38.	L1 (41)2	*48. Gigaspora*	7	Spores of the genus Gigaspora are yellowish to bright yellow with the characteristic of a bulbous suspensor. Spores are 350-360µm in size.
		49. Acaulospora	15	Spores of the genus Acaulospora are transparent and round with a diameter of 110-150µm; there are ornaments on the spore wall.

Table 6. Density analysis and identification of AMF spores to the morphospecies level, pepper production land of the Margo Utomo Farmer Group, Kedarpan Village, Kejobong District, Purbalingga Regency.

No.	Sample Code	Genus	Number of spores per 10 g	Description
1.	L2 (7)	21. *Glomus*	26	Spores of the genus Glomus are yellow and oval in shape, with a diameter of 70-150µm, forming only a spore wall.
2.	L2 (17)	22. *Glomus*	1	Spores of Glomus are yellowish with a diameter of 100µm; spores form spore walls.
		23. *Gigaspora*	3	Gigaspora spores are yellowish with a diameter of 200µm, but only the skin (spore-wall) is found.
3.	L2 (19)	24. *Glomus*	12	The genus Glomus's spores are yellow and round with a 120-130µm diameter. The formation of only spore walls characterizes spores.
4.	L2 (20)	25. *Gigaspora*	1	Gigaspora spores are clear with a diameter of 200µm. Spores form 2 walls (spore-wall and germinal wall), and there is also a bulbous suspensor with apparent hyphae.
5.	L2 (29)	26. *Scutellospora*	2	There is a germination shield and bulbous suspensor in the Scutellospora genus with clear spores, 380µm in diameter.
6.	L2 (31)	27. *Glomus*	2	Spores of the genus Glomus are clear in color with a diameter of 130x130µm. Spores only form spore walls without ornamentation.
7.	L2 (40)	28. *Entropospora*	4	Spores are clear with a diameter of 100µm. Spores form 2 walls (spore-wall and germinal-wall)
8.	L2 (21)2	57. *Glomus*	315	Glomus spores are yellowish, small round with a diameter of 70-110x70-110 µm; there is only a spore wall on the spores.
9.	L2 (5)2	58. *Gigaspora*	8	Gigaspora spores are clear, with a diameter of 300-350 pm, and there is a bulbous suspensor and two types of walls.
			18	Gigaspora spores are yellow, large, and round, with a diameter of up to 600 pm; there is a bulbous suspensor and two layers of walls.
10.	L2 (6)2	59. *Gigaspara*	3	Gigaspora spores are yellow and round with a diameter of 400-410 pm; there is a suspensar bulb, apparent hyphae, and two layers.
11.	L2 (23)2	60. *Glomus*	425	Spores of the genus Glomus that grow densely in the roots are yellowish, round, and small, with a diameter of

Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 3 No. 2 October 2024

				60-100 pm; spores only form spare walls, and the typical characteristics of Glomus are visible on the subtending hyphae.
12.	L2 (25)2	61. *Glomus*	169	Spores of the genus Glomus are yellowish with a diameter of 140 pm.

Table 7. Density Analysis and identification of AMF spores to the morphospecies level, pepper production land owned by residents of Kedarpan Village, Kejobong District, Purbalingga Regency

No.	Sample Code	Genus	Number of spores per 10 g	Description
1.	L3 (4)	50. *Glomus*	4	Glomus spores are yellowish round in shape, with a diameter of 100µm. In spores, there is only a spore wall.
2.	L3 (7)	51. *Glomus*	2	Glomus spores are yellowish round with a 90-110µm diameter. There is only a wall on the spores.
3.	L3(2)2	52. *Entrospospora*	4	Spores from the genus Entrospospora are round, yellowish in color, with a diameter of 100 µm. There are two walls (spore-wall and germinal-wall); there are no ornaments on the surface of the spore-wall. A saccule is seen in the opposite position to the subtending hyphae.
4.	L3 (7)2	53. *Gigaspora*	22	Gigaspora spores are yellow with a diameter of 100-350 µm; there is a visible bulbous suspensor.
5.	L3 (8)2	54. *Gigaspora*	3	Gigaspora spores are clear oval in shape, with a diameter of 190-200x230-270µm. There is a suspensor bulb, and spores form 2 walls; the hyphae are bright/apparent.
6.	L3 (9)2	55. *Gigaspora*	3	Gigaspora spores are clear oval in shape with a diameter of 250x270 µm; spores form 2 walls, and suspensor bulbs, bright/transparent hyphae.
7.	L3(10)2	56. *Glomus*	275	Glomus spores are yellowish, small, and round in shape with a diameter of 100-120µm; there are spore walls and typical structures in the hypha attachment.

External hyphae are other structures of mycorrhiza that develop outside the roots, while internal hyphae are parts of the hyphae that enter the root cortex cells and form a distinctive oval-shaped structure called vesicles and a hyphal branching system called arbuscules—external hyphae function to absorb nutrients and water in the soil. The presence of external hyphae associated with plants will play an essential role in expanding the root adsorption area, allowing the roots to absorb nutrients and water in a further range (Khairiyah et al., 2022).

Table 8. Density Analysis and identification of AMF spores to the morphospecies level from 3 pepper planting areas in Kedarpan Village, Kejobong District, Purbalingga Regency

No	Sample	Pepper nursery land	Pepper production land of the Margo Utomo Farmer Group	Pepper production land owned by residents
1.	Number of Mycorrhizal Samples	38	14	7
2.	Number of Samples with genus Glomus	32	7	3
3.	Number of Samples with genus Acaulospora	5	-	-
4.	Number of Samples with genus Gigaspora	3	5	3
5.	Number of Samples with genus Entrospora	1	1	1
6.	Number of Samples with genus Scutellospora	-	1	-
7.	The average number of spores/10g Glomus	80,41	16,66	21,61
8.	The Average number of spores/10g Acaulospora	11,61	0	0
9.	The Average number of spores/10g Gigaspora	5,70	0,57	2,15
10.	The Average number of spores/10g Entrospora	0,01	0,07	0,30
11.	The Average number of spores/10g Scutellospora	0	0,03	0

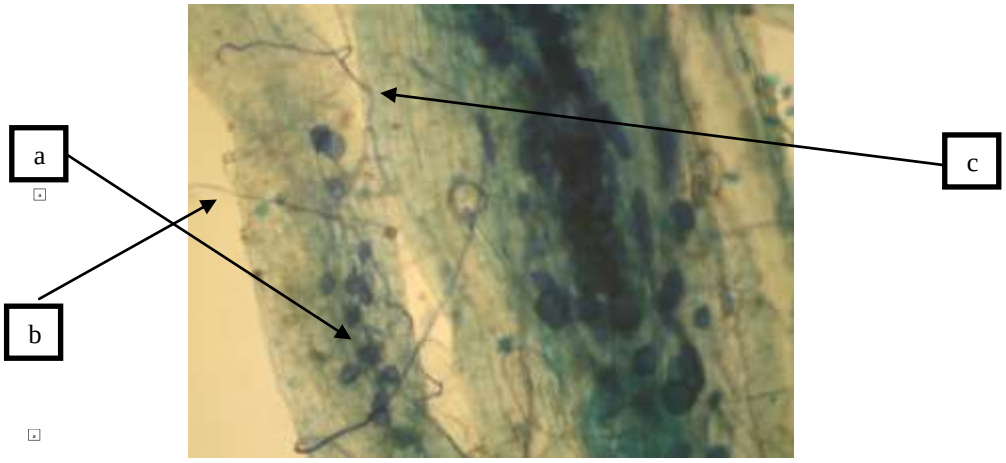

Figure 6. Roots Infected by Mycorrhiza. (a) Vesicles (b) External Hyphae and (c) Internal Hyphae in Roots with 40x Magnification

Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 3 No. 2 October 2024

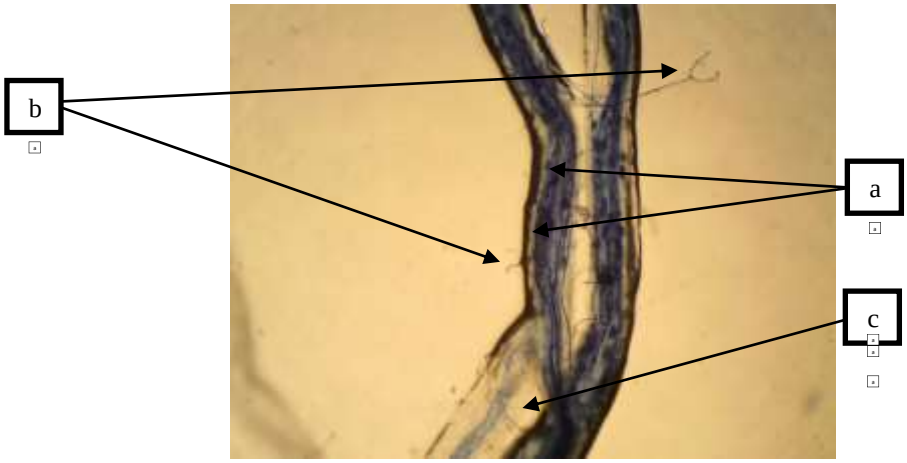

Figure 7. Roots Infected by Mycorrhiza. (a) Vesicles (b) External Hyphae and (c) Internal Hyphae in Roots with 10x Magnification

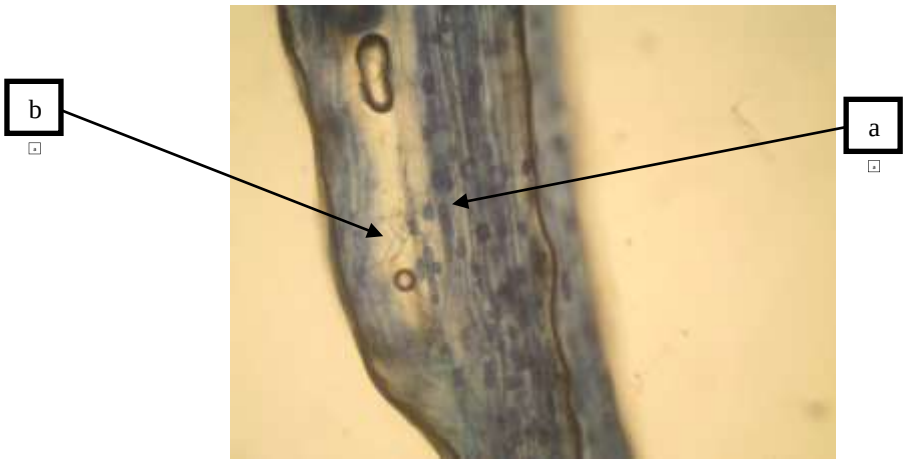

Figure 8. Roots Infected by Mycorrhiza. (a) vesicles, (b) Internal hyphae in Roots with 10x Magnification

Spore Population in Three Locations of Kedarpan Village, Kejobong District

Based on the results of research from three lands in Kedarpan Village, Kejobong District, the number of AMF spores found per 10 grams of propagation media can be seen in (Table 5), (Table 6), and (Table 7) is the analysis of Density and identification of AMF spores to the morphospecies level, it can be seen that each soil sample has a different number of AMF spores. The difference in the number of spores is thought to be due to differences in soil conditions and content, as stated by Sumba & Adiartayasa (2014), that many factors including physical and chemical conditions of the soil, influence the distribution of mycorrhiza. The study results show that from three different lands, the nursery land shows the highest number of spores seen (Table 4.1.), and the average level of spores is 80.41 per 10 grams (Table 3). Overall, it was also found that more AMF spores were found in the nursery land than in the other two lands—this is thought to be due to the type of spores found at the research location, namely the genus Glomus, as stated by Vallejos-Torres et al., (2020) that Glomus spores have an average spore size of 50-100 µm so that more were found on the 63 µm diameter sieve.

Based on data from each genus in the field, the genus Glomus has more spore types than the genera Gigaspora, Scutellospora, Acaulospora, and Entropospora. Glomus is a type of AMF that has the most dominant distribution. The genus Glomus germinates faster because the smaller spore size causes the hydration phase to occur very quickly, so the enzyme activity related to the germination process will take place faster (Kurnia and Gusmiaty, 2019).

In line with the results of research on the presence of AMF spores, as stated by Gusmawartati et al. (2014), who studied AMF in coastal forests also concluded that Glomus is the most dominant type of AMF in its distribution, namely 25 species out of 37 species found are of the genus Glomus.

Intensity of Root Infection by AMF

In general, the level of infection or colonization of AMF in pepper plantations in the three surveyed locations showed a high level of infection (> 30%). The level of AMF infection intensity in three different locations ranged from 80% -90%. The highest intensity of AMF infection in pepper plant roots was found in land 1, namely the nursery land (Table 4). However, the intensity of root infection by AMF in pepper plantations in each land did not show any significant difference in the intensity of root infection by AMF ($F = 6.114$; $p = 0.003$) based on the calculation of single factor ANOVA in the table (Table 4).

The presence or absence of roots can show the association between AMF and a plant infection. AMF infection can be identified by the presence of infection structures produced by AMF, including hyphae, mycelia, vesicles, and arbuscules. The infection studied in the roots of pepper plants found infection structures that form internal hyphae, external hyphae, and vesicles (Figure 1). Various factors, including fertilization, plant nutrition, pesticides, light intensity, season, soil moisture and pH, high inoculum density, and plant information levels, influence AMF infection intensity. The formation of vesicle and arbuscule structures in the roots of pepper plants in Kedarpan Village shows that plants can form a symbiosis with AMF and that AMF infection has occurred in the roots of pepper plants. As age increases, these arbuscules change into a clumped structure, and the arbuscules' branches can no longer be distinguished. Duddridge and Read (1982) reported that one plant root can be infected with more than one type of AMF and vice versa; one type of AMF can infect more than one plant root.

Arbuscular and vesicular structures are specific structures formed by AMF, which are significant in identifying that AMF infection has occurred in plant roots. According to (Brundrett et al., 1996), the formation of arbuscules begins with the entry of internal hyphae from AMF and grows between the cortex cells, then penetrates the host cell wall and develops inside the cell. Meanwhile, according to Abbott and Robson (1982), vesicles originate from the swelling of internal hyphae from AMF, and vesicles are found inside and outside the parenchymal cortex layer.

The development of AMF colonization begins with the formation of an appressorium on the root surface by external hyphae originating from germinating spores. The appressorium enters the root through the gap between the epidermis, then forms intracellular hyphae along the root epidermis, after which arbuscules and vesicles are formed (Nainggolan et al., 2014).

Characterization and Identification of Mycorrhizal Fungi

The results of AMF identification on pepper plants in Kedarpan Village, Kejobong District, Purbalingga Regency have many characteristics. The diversity of spores obtained is

distinguished based on the shape of the spores, ranging from round, oval, and irregular. The shape and color of the spores' size describe each spore's characteristics. The description of each AMF that was successfully isolated is as follows.

Glomus Genus

Based on Table 8, the Glomus genus was found in three fields in Kedarpan Village, Kejobong District. Based on morphological identification (shape and color), the AMF genus found from the Glomus genus has characteristics of a round to oval shape, spore color ranging from clear, yellow to brownish, and spore walls consisting of two layers of spore walls. As in (Figure 1) and (Table 5) the spores of Arbuscular Mycorrhizal Fungi of the genus Glomus in the pepper nursery land of Kedarpan Village, Kejobong District, Purbalingga Regency, show a yellowish color, round shape with a diameter of 100-120 µm, forming a characteristic of Glomus in its subtending hypha—this is reinforced by the criteria according to the International Culture Collection of Vesicular Arbuscular Mycorrhizal (INVAM, 2017), which has a round shape. The spore wall consists of more than one layer. The color of the spores varies from yellow, brownish yellow, yellowish brown, and light brown to dark brown and blackish.

According to Widitama (2015), Glomus spores are round and oval and have a reasonably wide distribution; the spore wall consists of four layers, does not react with Melzer's solution, has no ornamentation, the hypha seat (subtending hypha) of Glomus is cylindrical and straight with an average size of 259 µm.

Genus Gigaspora

Table 8 and Figure 2 show that the genus Gigaspora was found in three Kedarpan Village Kejobong District fields. The genus Gigaspora found in several research fields has the following characteristics: round, yellow, and tailed with large to enormous sizes, diameter of 270-420 µm, there is a bulbous suspensor with bright transparent hyphae.

According to the International Culture Collection of Vesicular Arbuscular Mycorrhizal (INVAM, 2017), the genus Gigaspora is characterized by its distinctive characteristics of having a bulbous suspensor, relatively large in size and round in shape, the color of the spores varies from yellow, greenish-yellow, brownish yellow to yellowish brown.

Gigaspora spores react with Melzer's solution thoroughly and have no ornamentation. Gigaspora hyphae form a bulbous suspensor or rounded hypha seat. Gigaspora has auxiliary cells/additional cells that embody external vesicles. The characteristics of Gigaspora spores are that they are bright yellow in color, round in shape with an average size of 321µm, and their walls consist of three layers (Widiatma, 2015).

Genus Scutellospora

Based on Table 8 and Figure 3, the genus Scutellospora was found in the KT production land of Kedarpan Village, Kejobong District. The genus Scutellospora found in several research lands has a round shape, with clear spores, two layers of spore walls, a diameter of 380µm, and a germination shield and bulbous suspensor.

According to the International Culture Collection of Vesicular Arbuscular Mycorrhizal (INVAM, 2017) the genus Scutellospora is characterized by having a thin spore wall layer of approximately 1-2 layers, reacting with Melzer, having ornaments in the form of a germination-shield, hyphae forming a bulbous suspensor or hypha seat that wraps, has

auxiliary cells that can be said to be the manifestation of external vesicles, has a reddish brown spore color, pale yellow to yellow-brown in older spores. The shape varies, from subglobose to ellipsoid to oval, sometimes irregular (INVAM, 2017).

The development process of Scutellospora is the same as Gigaspora. Gigaspora spores and Scutellospora spores differ in the germination shield's germ layer. When Scutellospora spores germinate, hyphae come out of the germination shield. Scutellospora sp. is a genus of mycorrhizae included in the Gigasporaceae family. This genus has several characteristics, including spores with or without decoration, spores consisting of flexible spore walls, and spore structures that are ovoid, obovoid, pyriformis, or irregular. The process of spore formation in Scutellospora sp. is the same as the formation of spores in the genus Gigaspora sp. The difference between the genus Gigaspora sp. and Scutellospora sp. is that in Scutellospora sp., there is a germination shield (Ramadhani et al., 2019).

Genus Acaulospora

Based on Table 8, the genus Acaulospora was only found in the pepper nursery land of Kedarpan Village, Kejobong District. Acaulospora spores are characterized by being transparent in color and round in shape with a 140-150µm diameter. Spores form 2 walls, namely the spore wall and the germinal wall. On the surface of the spore wall, distinctive ornaments characterize the species, as in Figure 4.

According to the International Culture Collection of Vesicular Arbuscular Mycorrhizal (INVAM, 2017). The genus Acaulospora is characterized by an irregular globus subglobus that is elliptical. The spore wall consists of two layers, and the innermost spore wall is equipped with a germination orb. The color of the spores varies from yellow, orange, brownish, and dark red to brownish red.

The development process of Acaulospora spores seems to be from hyphae, but it is not. The development process begins at the end of the hyphae seat, which enlarges like a spore called the terminal hypha (hyphal terminus). Small circles that gradually enlarge and become spores are formed between the hyphal terminus and the subtending hyphae. The terminal hyphae will be damaged in its development, and its contents will enter the spore. Damage to the terminal hyphae will leave a small hole called a cicatrix. The characteristics of Acaulospora spores are that they are predominantly red, the walls consist of three layers, are ornamented, and the average spore size is 279 µm (Widiatma, 2015).

Genus Entrospora

Table 8 shows that Entrospora was only found in the pepper nursery land of Kedarpan Village, Kejobong District. Entrospora spores found in the land have the characteristics of being round and yellowish in color with a diameter of 100 µm. There are two walls (spore-wall and germinal wall). There are no ornaments on the surface of the spore wall. There is a saccule with a position opposite to the subtending hyphae.

According to the International Culture Collection of Vesicular Arbuscular Mycorrhizal (INVAM, 2017). The Entrospora genus is characterized by having a round shape, yellowish in color, with a diameter of 100 µm. There are two walls (spore-wall and germinal-wall), and there are no ornaments on the surface of the spore-wall. There is a saccule with a position opposite to the subtending hyphae.

In line with the results of the FMA study on the Entrospora genus, as reported by the Entrospora genus, it has a yellow-brown spore color; if the spores are not yet mature, the color looks much more opaque. Spores are round with an average size of 121 µm. The

spore wall consists of two layers; in the study conducted, the Entrophospora sp. species was found (Ramadhani et al., 2019).

The Influence of Several Land Characteristics in Three Locations of Kedarpan Village, Kejobong District on Arbuscular Mycorrhizal Fungi

The presence of AMF can be influenced by several factors, such as soil factors (humidity, supporting capacity, temperature) and plant factors (root length, root density, and root infection). In pepper cultivation in Kedarpan Village, Kejobong District, Purbalingga Regency, pepper farmers use an organic system without using chemical fertilizers or pesticides; according to (Widiati et al., 2015), soil texture affects the development and growth of soil spores which tend to be clayey mud suitable for the development and growth of Glomus spores, so that the Glomus genus is more commonly found in agricultural land in Kedarpan Village, Kejobong District.

Based on the results of observations of the shape of the spores, the number and genus found in each location vary. This condition indicates the diversity of AMF found in each land. The diversity in the genus and number of spores found can be caused by differences in location characterized by differences in soil processing; this is in line with the statement of Budi & Dewi (2016) that differences in location and rhizosphere can cause the diversity of AMF species and populations.

In 2021, pepper farmers in Kedarpan Village applied mycorrhiza to nursery land; this is evident in Table 8, where the number of mycorrhizal samples was higher than in farmer group production land and residents' production land. The colonization and proliferation of AMF involve spore production, and spores are responsible for spreading AMF to the soil environment. AMF can form associations with plant roots, which can expand the root surface and facilitate nutrient absorption; it is believed that AMF can produce compounds that stimulate plant growth and facilitate nutrient transport through the hyphal network (Dharmaputri et al., 2016).

Based on the study's results, different numbers of AMF populations were also found in other lands. The AMF genera Glomus, Gigaspora, Acaulospora, and Entropospora were found in the nursery land. However, the genus Scutellospora cannot be found on farmer group production land or residents' production land. The genus Glomus can spread to three research areas because of Glomus. According to (Kurnia et al., 2019), soil dominated by clay fractions is considered suitable for developing Glomus spores, while sandy soil is ideal for developing Gigaspora.

AMF develops at stable humidity and water content, not too high or too low. If the water and humidity levels are very high or excessive, it can cause anaerobic conditions that inhibit the development of mycorrhizae because all mycorrhizal-forming fungi are obligate aerobic. Meanwhile, low soil water content causes drought-resistant conditions. Dry land is very supportive of the development of mycorrhizae, where the low availability of nutrients in dryland conditions optimizes the growth of mycorrhizal hyphae (Mandjarara et al., 2019).

CONCLUSION

Arbuscular Mycorrhizal Fungi in pepper plantations in Kedarpan Village, Kejobong District, Purbalingga Regency that were successfully isolated and identified contained five genera of fungi, namely Gigaspora, Glomus, Acaulospora, Scutellospora, and Entropospora with morphospecies diversity in each genus. Glomus was found in all locations where

rhizosphere samples were taken. In the pepper nursery land, there were the genera Glomus, Gigaspora, Acaulospora, and Entrophospora; the pepper production land of the Margo Utomo Farmers Group had the genera Glomus, Gigaspora, Scutellospora, and Entrophospora. In contrast, the pepper production land owned by residents had Glomus, Gigaspora, and Entrophospora.

In general, the level of infection or colonization of AMF in pepper plantations in the three locations observed showed a high level of infection (>30%). The level of AMF infection intensity in three different locations ranged from 80% - 100%. The highest intensity of AMF infection in pepper plant roots was found in land 1, the nursery land. However, in each pepper plantation area, AMF did not significantly affect the intensity of root infection ($F = 6.114$; $p = 0.003$). The diversity and density of the Arbuscular Mycorrhizal Fungi population in pepper plantations have the potential to be developed as biological agents to control stem rot disease caused by Phytophthora sp. fungi.

ACKNOWLEDGEMENTS

We want to thank PT Java Agritech (Ladatech R&D), Semarang, Indonesia, as part of this work funded.

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