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Study on the Population and Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of *Piper nigrum* in West Lampung Indonesia

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ABSTRACT. The population and diversity of arbuscular mycorrhizal fungi (AMF) in soil vary greatly, influenced by biotic and abiotic factors. West Lampung Regency is a major prpper production center with distinct biotic and abiotic factors. These differences affect the diversity of AMF in pepper cultivation areas in West Lampung Regency. This study aimed to determine the differences in the population and diversity of indigenous AMF in the rhizosphere of pepper plants grown in monoculture and mixed cropping system. Soil sample were collected from monoculture and mixed pepper plantations. The AMF population in the samples was determined by isolating AMF spores from the soil using the wet sieving method. Trap cultures were established using soil samples from each plantation, using maize as the host plant, for duration of 3 months. The results showed that the AMF population in the rhizosphere of mixed pepper plantation was higher than that in monoculture plantations. Based on trap culture, 9 AMF types (S1, S2, S3, S4, S5, S6, S7, S8, and S9) were identified in mixed cropping system, while 5 AMF types (S4, S5, S6, S7, and S9) were found in monoculture plantations.

Keywords : *pepper, monoculture, mixed cropping*

INTRODUCTION

The population and diversity of arbuscular mycorrhizal fungi (AMF) in soil vary greatly and are influenced by several factors, including biotic and abiotic factors. Biotics factors include the type of mycorrhizae and host plants, while abiotic factors encompass soil fertility, temperature, water availability, and pH. Limited water availability for host plant growth can stimulate AMF activity, encouraging the fungi to expand their reach by forming more extensive mycelial network (Souza, 2015). Reduced water availability also decreases nutrient solubility, prompting AMF to become more active in nutrient absorption to meet the requirement of the host plant (Indriana et al., 2020). Light intensity id another critical factor influencing AMF activity. Higher light intensity enhances the rate of photosynthesis in leaves, resulting in increased assimilate allocation to the roots. This process provides AMF with sufficient energy to support their growth and development (Aryanto et al., 2018; Rini et al., 2020).

Several studies have highlighted that greater plant species diversity can enhance the abundance of AMF spores. Guzman et al. (2021) found that diverse host plants drive a shift in AMF communities toward greater richness and diversity, emphasizing that plant diversity plays a crucial role in enriching AMF populations. Simanungkalit (2004) explained that host plants with extensive root systems are more responsive to AMF inoculation, as such root systems promote the formation of association with AMF and support sporulation (Gunawan, 1999).

Another factor influencing the population and diversity of AMF is soil pH and condition. Nurhayati et al. (2012) stated that the optimal pH range for AMF development is between 3.9 and 5.9. in addition, low soil nutrient levels, particularly phosphorous, can increase AMF colonization in plat roots, as AMF plays a crucial role in phosphorous absorption. Pulungan (2013) explained that high phosphorous availability in soil directly reduces AMF activity, leading to decline in AMF presence. Conversely, when soil phosphorous levels are low, AMF colonization tends to increase because plants rely more on AMF as a mechanism to acquire nutrients under such condition (Palasta and Rini, 2017).

Pepper (*Piper nigrum* L.) is an important crop in Indonesia due to its high economic value, making it one of the country's sources of foreign exchange. Pepper cultivation is typically carried out by smallholder farmers and requires significant labor input (Manohara et al., 2006). Pepper farming in Indonesia depends on the characteristics of the pepper varieties cultivated, the profile of pepper farmers, and environmental factors. As a result, pepper cultivation in Indonesia can be categorized into conventional farming, pesticide-free farming, and organic farming, with both monoculture and mixed cropping planting systems, each having its own advantages dan disadvantages (Prasmatiwi and Evizal, 2020).

The main pepper production areas in Indonesia are located in seven provinces: South Sumatra, Bangka Belitung, Lampung, East Kalimantan, South Sulawesi, and Southeast Sulawesi. The area of pepper cultivation in Indonesia has increased over the past three years, from 189,703 hectares in 2019 to 190,452 hectares in 2020, and 193,388 hectares in 2021, This expansion has been accompanied by a rise in pepper production during the same period, with yield of 87,619 tons in 2019, 88,254 tons in 2020, and 89,153 tons in 2021 (Direktorat Jenderal Perkebunan, 2021). Currently, Lampung Province ranks as the second-largest pepper producer in Indonesia, following Bangka Belitung Province. The total pepper cultivation area and production in Lampung are 48,847 hectares and 14,689 tons, respectively (Direktorat Jenderal Perkebunan, 2021).

The increase in pepper plant production is achieved through proper cultivation techniques, particularly in the use of fertilizers. Farmers still heavily rely on inorganic fertilizers to maintain their crops. Farmers in West Lampung, especially in Pekon Talang Jaya, are highly dependent on the use inorganic fertilizers such as Mutiara fertilizer. The use of inorganic fertilizer has positive impacts on plants, as fertilizers like NPK contribute essential nutrients-Nitrogen (N), phosphorous (P), and potassium (K)-to the soil, which are crucial for plant growth. Phosphorous is an essential macronutrient required by plants; however, it is not easily soluble, with only 15-20% being absorbed by plants while the rest is retained in soil colloids (Ginting et al., 2006). On the other hand, inorganic fertilizers also have negative impacts, including altering soil structure, causing compaction, depleting soil nutrient content, and contributing to environmental pollution. Continous use of inorganic fertilizers can also increase soil acidity, which negatively affect soil microorganisms. If this contionus over time, it can lead to a decline in the soil's natural fertility (Triyono et al., 2013).

The pepper cultivation practices in Pekon SIndang Pagar, Sumberjaya District, West Lampung Regency, utilize a mixed cropping system. This system involves cultivating two or more different primary crops on the same plot of land. In this case, the mixed cropping system in Pekon Sindang Pagar, the famers use pepper and coffee as the primary crops. In contrast, the cultivation practices in Pekon Talang Jaya, Sumberjaya District, West Lampung employ a mococulture cropping system which involves cultivating only one type of primary crop, which is pepper. Beside the main or primary crops, the farmer also plant shade trees to provide necessary shading to the pepper plants. The differences in cultivation practices between Pekon Sindang Pagar and Pekon Talang Jaya affect the population and diversity of mycorrhizal fungi (AMF) in the pepper cultivation areas. Therefore, the objective of this study was to determine the differences in the population and diversity of indigenous AMF in the rhizosphere of pepper plants grown in monoculture and mixed cropping system.

MATERIALS AND METHODS

This research was conducted in the greenhouse and Plantation Crop Production Labaratory of the Faculty of Agriculture of Lampung University from November 2022 to May 2023.

Soil Sampling

Soil samples were taken from the rhizosphere of pepper plant grown in mixed cropping with coffee (Pekon Sindang Pagar) and monoculture (Pekon Talang Jaya) using purposive sampling technique. In mixed cropping system, Jambi Variety pepper plants were planted together with Ciari variety of Coffee, while in the monoculture system, only Kerinci variety pepper plants were cultivated with shade trees. Seven sample points were determined for each farm. At each sample point, soil samples were taken from three pepper plants, and for each plant, soil samples were collected from two different places. Soil samples were taken to a depth of 20 cm using a soil auger. The soil samples were then mixed together to represent one soil sample. The total number of soil samples was 14, coming from two farms (mixed cropping and monoculture), each weighing approximately 2,5 kg. The collected soil samples were used to calculate the AMF population and for carrying out trapping culture to determine the diversity of AMF species.

Determination of AMF Spore Population

The isolation of the AMF spore from soil was done using wet sieving method. The isolated spores were counted manually with the help of a stereo microscope. The data obtained were then tested using a T-test to determine if there were significant differences in the AMF population between the soil sample from two cropping systems of pepper.

Trapping Culture

The planting media used are sand, zeolite, and soil sample from pepper plantations that have been composited (seven soil samples from each pepper cultivation system were composites into one soil sample). The sand was sterilized twice at a temperature of 120 °C and a pressure of 1 atm for 60 minutes, then washed with running water. Zeolite used as planting media was also washed thoroughly with running water beforehand. The planting media is a mixture of sand and zeolite in a ration 2:1 (volume:volume). The sand and zeolite were mixed and stirred until homogenous, then placed into polybags measuring 14 x 20 cm with approximately 700 g of the mixture.

The host plant used for trapping culture was maize. Before planting, the seeds are soaked in a 10% Clorox solution for 15 minutes to sterilize them. The seeds are then germinated by placing them on moistened straw pepper and storing them at room temperature for three days while maintaining adequate humidity.

The composited soil sample, weighing approximately 250 g, was placed into polybags that already containing 700 g of the sand and zeolite mixture. The pre-germinated host plant seeds are planted on top of the soil sample at density of 5 seedlings per polybag, then covered again with the sand and zeolite media (Figure 1)

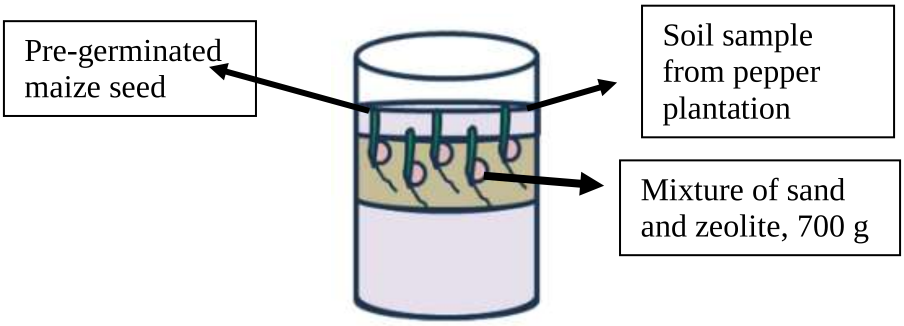

Figure 1. Illustration of trapping culture

The trapping culture was maintained in a greenhouse for 3 months. Plant maintenance includes fertilization, watering, weeding, pest and disease control, and flower pruning. The fertilizer used are Urea and NPK. Urea fertilizer is applied twice a week starting from the plant's age of 2 weeks until 6 weeks, using a concentration of 2 g/L and a dose of 20 ml per polybag. Meanwhile, NPK fertilizer was applied when the plants were 6 weeks old at a dose of 0.3 g per polybag. Watering was done daily, but no watering is performed during the last 2 weeks before terminating the culture, allowing the plants to dry out. Weeding was conducted manually pulling out weeds growing in the polybags. Pest control was carried out manually by capturing and eliminating pests. Flower pruning was done using scissors.

At the end of the study, the polybags were cut to separate the upper and lower sections of the planting media. The lower section of the media (700 g of mixture of sand and zeolite) was used to count the population and diversity of AMF. AMF spores produces from the host plant roots were isolated using wet sieving technique and manually counted under stereo microscope. The spores were subsequently identified by observing their color (based on Cayan, Yellow, Magenta color chat), size, shape, and the presence or absence of spore ornament such as bulbose suspensor, sporiferous saccule, cicatrix, and spore reaction to the Melzer solution. AMF root colonization was determined by staining pepper root sample using ink solution and the stained roots were observed under a compound microscope, and the percentage of root colonization was calculated based on the number of observation showing AMF structures divided by the total number of observations.

RESULTS AND DISCUSSION

Based on the results on the T-test (Table 1), it was found that the AMF spore population in soil samples from mixed cropping system was significantly higher that the AMF population in soil samples from monoculture pepper plantations. Data analysis using T-test indicated a P-value ≤ 0.01. meaning there was a significant different at α 1%. The difference in spore counts between mixed cropping and monoculture plantation may attributed to differences in fertilization practices. The farmers in mixed cropping plantation used organic fertilizer derived from coffee grounds and goat manure. In contrast, the farmers in monoculture plantation used inorganic NPK fertilizer. Research by Ratnawati et al. (2016) showed that treatment without nitrogen fertilizer had higher AMF spore counts compared to those with added nitrogen fertilizer. In addition, Puspitasari et al. (2012) stated that high nitrogen levels can inhibit AMF spore development, as plants becomes less dependent on AMF symbionts.

Table 1. T-test results for AMF population in the rhizosphere of pepper in mixed and monoculture plantations

	Plantation	Mean (AMF/50 g soil)	P
Value	Mixed Cropping	41,71	0,00
	Monoculture	23,57	

Note: P = Prabability value

For AMF root colonization, both in the mixed cropping and monoculture cropping systems, the AMF colonization percentage was very low, below 10% (Table 2). This in line with the statement by Soemartiningsih et al. (2015), who suggested that AMF colonization percentage in the range of 0-25% are categorized as low, 28-50% as moderate, 51-75% as high, and 76-100% as very high.

Table 2. AMF colonization on pepper roots from mixed cropping and monoculture plantation

Plantation	Replication (%)							Total	Means
	1	2	3	4	5	6	7		
Mixed cropping	0.9	0.42	1.11	0.45	0.77	0.98	0	4.63	**0.66**
Monoculture	0.64	5.38	1.83	4.44	8.4	2.05	0	22.74	**3.25**

Cultivation system influenced the number of AMF type present in the soil. Results in Table 3 showed that more AMF type found in the rhizosphere of pepper from mixed cropping system compared to monoculture plantation. The characteristics of each type of AMF are presented in Table 4.

Table 3. AMF spore type from trapping culture using soil sample from rhizosphere of pepper planted in mixed cropping and monoculture cultivation system

Plantation	Spora/ 400 g media	Σ AMF Type	Description
Mixed cropping	745.4	9	S1, S2, S3, S4, S5, S6, S7, S8, S9
Monoculture	1096.4	5	S4, S5, S6, S7, S9

The cropping system used can also influence the diversity of AMF. In mixed cropping system, pepper plants are intercropped with coffee, while in monoculture plantations, only pepper plants are grown as the main crop. According to Guzman et al. (2021), the diversity of host plants can shift the existing AMF community into richer and more divers community. This is consistent with the statement of Giovanni et al. (2020) and Xu et al. (2017), who said that biodiversity always varies by location. This is due to environmental factors, which are a combination of various physical, chemical, and biological processes that can influence the life of organisms.

Table 4. Characteristic of AMF types from rhizosphere of pepper

Description	**S1**	**S2**	**S3**	**S4**	**S5**
Spore size	274 µm	302.6 µm	228.4 -234.1 µm	107-129.5 µm	172-194 µm
Color (C,Y, M)	0, 50, 0	0, 20, 0	20, 20, 20	20, 100, 60	0, 40, 0
Sporocarp (S)	None	None	None	None	None
Sporiforeous saccule (SS)	None	None	None	Present	None
Bulbose suspensor (BS)	Present	Present	Present	None	Present
Germination shield	None	Present	None	None	None
Melzer reaction	Positif	Positif	Positif	Positif	Positif
	S6	**S7**	**S8**	**S9**	
Spore size	148.4-188.4 µm	137-166 µm	154-177 µm	63-69 µm	
Color	0, 20, 0	20, 100, 60	0, 20, 0	0, 50, 10	
Sporocarp (S)	None	None	None	None	
Sporiforeous saccule (SS)	None	Present	None	Present	
Bulbose suspensor (BS)	Present	None	Present	None	
Germination shield	None	None	None	None	
Melzer reaction	Positif	Positif	Positif	Positif	

The pictures of some AMF type from this study are presented in Figure 2.

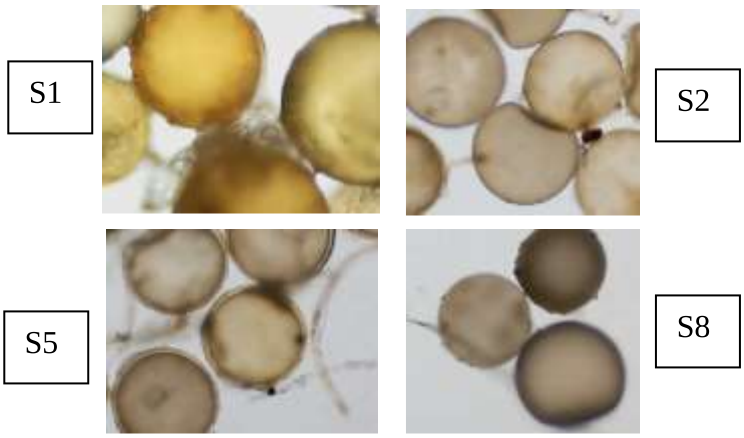

Figure 2. The pictures of AMF type S1, S2, S5, and S8.

CONCLUSION

Based on the results obtained in this study, it can be concluded that the population and diversity of indigenous AMF in the rhizosphere of pepper grown in a mixed cropping system were higher than those in a monoculture cropping system.

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