

Trapping Cultures For Arbuscula Mycorrhizy Fungi (AMF) Spores Trapping From Post-Mine Asphalt Soils Using Different Host Plants

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ABSTRACT. Spore trapping Culture has the advantage of producing AMF. inoculum production and higher A.M.F. colonization efficiency. This research aims to determine suitable hostnames for AMF trapping. This research was conducted at the Plastic House of the Indonesian Mycorrhizal Association (AMI.) Southeast Sulawesi Branch, UHO Old Campus, and the UHO. Forestry and Environmental Science Laboratory Unit lasted 3 months from May – June 2023. This research used a factorial, completely randomized design method with two factors: the first factor is a type of location, including L10 LM10, and the second factor is the type of host plant, including *Pueraria javanica* and *Sorhum bicolor*. The results showed that the interaction of the host plant and location had no significant effect on the number of spores and AMF colonization. *Pueraria Javanica* significantly increased the number of spores for both L10 and LM10, with an average number of spores of 84. Seven dominant types of A.M.F. from the Glomeraceae family were found

Keywords : *Glomus rubiforme*, *Pueraria javanica*, *Aspal*, *Restoration*

INTRODUCTION

Mycorrhiza is a symbiosis between soil fungi and plant roots (Nurbaiti et al., 2009). Mycorrhiza is divided into 2, namely endomycorrhiza and ectomycorrhiza. One group of endomycorrhiza is arbuscular mycorrhiza fungi (AMF.). AMF are soil microorganisms that are symbiotic with the roots of higher plants and are generally found in terrestrial ecosystems (Husna et al., 2014). Arbuscular mycorrhiza fungi are included in obligate fungi from the Glomeromycota phylum, where fungal hyphae enter plant cells that form highly branched structures called arbuscules (Husna et al., 2018; Kobayashi et al., 2018). AMF has also been reported to be used as mycorrhizal biofertilizer (Hajoeningtjas, 2009).

Studies of arbuscular mycorrhizal fungi in Southeast Sulawesi have been reported in post-nickel mining lands (Husna et al., 2015), gold (Tuheteru et al., 2020), and asphalt (Tuheteru et al., 2022). In post-asphalt mining lands, nine types of AMF have been found belonging to five genera, namely, *Claroideoglomus*, *Haloatospora*, *Glomus*, and *Gigaspora*, three families. *Claroideo*, *glomus* and *claroideum*. Research on A.M.F. diversity is still limited to field soil (Tuheteru et al., 2022). Spores collected from the field tend to have a low number of spores due to parasitism by soil fauna and bacteria and degradation of characteristics in the spore wall (Suting & Nongthombam, 2021). To overcome this difficulty, carrying out spore trapping Culture is necessary.

Pore trapping Culture has been widely used. Studies on AMF trapping have been conducted in limestone (Suting & Nongthombam, 2021), gold (Tuheteru et al., 2020), and coal (Makdoh and Kayang, 2019) mines. The spore trapping method has advantages in producing higher AMF inoculum production and AMF colonization efficiency (Selvakumar et al., 2018). This trapping Culture approach does not reveal the same AMF species community composition as direct spore analysis in the field; this difference is due to the selective effect of the host plant (Velazquez & Marta, 2011).

Sporulation of several AMF species is influenced by the host plants selected for spore trapping. Host plants used in spore trapping include *Zea mays* (Makdoh & Kayang, 2019), *Sorghum bicolor* (Tuheteru et al., 2020), and *Pueraria javanica* (Husna et al., 2015). Plants used as host plants for spore trapping have the following requirements: a short life cycle, adequate root system development, reasonable colonization rate, tolerance to relatively low phosphorus (P) levels, and low susceptibility to pathogens (Ijdo et al., 2010). AMF trapping from post-asphalt mining land, especially using different host plants, has not been done. This study was conducted to determine suitable host plants for AMF spore trapping from post-asphalt mining land.

MATERIALS AND METHODS

Research Design

This research will use a Factorial Completely Randomized Design (RALF). The location factors include L10 (10-year Lawele Over Burden IUP) and LM10 (10-year Lawele Post-Mining IUP). The second factor is the type of host plant, including *Pueraria javanica* and *Sorghum bicolor*.

Research Variables

Research Variables	Formula
Number of Spores	Spore density is calculated using the formula: number of spores/inoculum weight.
AMF colonization	$[\Sigma \text{ mycorrhizal field of view} / \Sigma \text{ total observed field of view}] \times 100\%^{**}$
Relative Abundance	Percentage of the number of spores of a species or genus *
Relative frequency	$[\text{Number of soil samples where AMF type or genus was found} / \text{total samples}] \times 100\%^{*}$
Importance value index	$(FR+KR)/2$. INP ≥ 20 including dominant type or genus*

Research Procedures

1.Preparation of culture

The river sand media is first washed clean to remove dirt. Unclean river sand can negatively impact the development of AMF. Then, the sand is sterilized with a sterilizer for 8 hours to remove any possible pathogens.

2.Host Plant Preparation

Sorghum bicolor and *pueraria javanica* used as host plants were first soaked in bayclin solution with 2ml/1 liter of water for 5 minutes, then rinsed with clean water 3 times. The seeds were soaked in water for ± 24 hours to break the dormancy that might occur. After that, the seeds can be planted directly in the Culture pot.

3.Multiplication of FMA

For each sample, prepare three pots containing asphalt post-mining soil. Move the germinated seeds into the pots. Place 50 g of asphalt post-mining soil mixed with river sand and Grow the plants in a greenhouse for 3 months. (Trejo, 2020).

4.Maintenance

Culture maintenance activities include watering twice a day with a 20 ml water volume. In addition to watering, other maintenance activities include weeding and cutting dead leaves or branches of the host plant. Every one (one) week, watering is carried out with Biohara fertilizer at a ratio of 2.5 ml/water.

Data analysis

Data were analyzed using variance analysis (F test). Suppose the test results show a significant effect. In that case, the treatment difference test will be continued according to the Duncan Multiple Range Test (DMRT) at a 95% confidence level using SAS 9.1.3 portable software. AMF ecological data were analyzed using descriptive analysis of all observed variables. The results of the analysis are presented in table form.

RESULTS AND DISCUSSION

Recapitulation of the results of the analysis of variance (F test)

The recapitulation of the analysis of variance (F test) of host plant type treatment and location factors on the observation variables is presented in Table 1. It shows that the interaction between location treatment and host type did not significantly affect the variables of the number of spores and AMF colonization. The treatment of the host plant type significantly affected the number of spores and significantly affected the root colonization variable.

Table 1. Recapitulation of variance analysis of the influence of host plant types and location factors on research variables.

Observation Parameters	l Location (A)	Host Plants (B)	A×B	cv
Number of Spores	tn	*	tn	21,16
Root colonization	tn	**	tn	26,91

Note: ($P \leq 0.01$) = Very significant effect (**), ($P \leq 0.05$) = Significant effect (*), ($P \geq 0.01$) and ($P \geq 0.05$) = No significant effect (tn). (A): Location, (B): Host plants. Coefficient: Variation (CV).

Spore number and AMF Colonization

There was no difference in the effect of location treatment on the number of spores and root colonization. However, the highest number of spores and root colonization was at location LM10. *Pueraria javanica* was significantly different from *Sorghum bicolor*. In the AMF colonization variable, *S.Bicolor* plants had the highest AMF colonization percentage (49.44%) compared to *P. Javanica* (35.71%).

Table 2. Average number of spores and root colonization at different locations

	Number of Spores	Root colonization (%)
Location (A)		
LM 10	66,17 a	82,0 a
L 10	36,50 a	62,0 a
Host Plants (B)		
<i>Pueraria javanica</i>	83,67 a	35.71 b
<i>Sorghum bicolor</i>	18.83 b	49.44 a

Note. The mean value of the variable and the comparative value of the Duncan's multiple range test (DMRT) of the same letter in the same column are not different at 95% confidence. LM 10 (IUP Lawele over burden 10 years old), L10 (LUP Lawele post-mining 10 years old)

Ecology AMF

Seven types of AMF from the Glomeraceae and Acaulosporaceae families were found. At site L10, six types were found: *Glomus rubiforme* had the highest INP on the *Pueraria javanica* host, and *Glomus* sp 1 had the highest INP on the *Sorghum bicolor* host. While at site LM10, *Glomus* sp 2 had the highest INP on the *Pueraria javanica* host, and *Glomus* sp 1 had the highest INP on the *Sorghum bicolor* host.

Table.4 Ecology of FMA Site L10

No	Type	<i>Pueraria javanica</i>			<i>Sorghum bicolor</i>		
		FR	KR	INP	FR	KR	INP
1	<i>Glomus</i> sp.1	100	9,3	54,6	66,7	33,8	49,9
2	<i>Glomus</i> sp.2	66,7	2,5	34,25	33,3	5,2	19,1
3	<i>Glomus</i> sp.3	66,7	3,4	34,71	66,7	6,5	36,25
4	<i>Glomus</i> sp.4	-	-	-	33,3	4,0	18,5
5	<i>Acaulospora</i> sp.1	33,3	5,4	19,2	33,3	13,0	23
6	<i>Glomus rubiforme</i>	33,3	79,4	56,2	-	-	-
Total		300	100	200	200	100	100

Tabel.5 Ecology of FMA LM10

No	Type	<i>Pueraria javanica</i>			<i>Sorghum bicolor</i>		
		FR	KR	INP	FR	KR	INP
1	<i>Glomus</i> sp.1	67,7	15.9	41,45	67,7	66	66,5
2	<i>Glomus</i> sp.2	67,7	23.3	45,15	33,3	13.2	23.1
3	<i>Glomus</i> sp.3	33,3	9.2	21,1	100	11.8	55,9
4	<i>Acaulospora</i> sp.1	33,3	38.2	35.6	33,3	68.4	50,7
5	<i>Glomus rubiforme</i>	33,3	2.1	17,55	-	-	-
6	<i>Glomus</i> sp.4	33,3	5.7	19,35	-	-	-
7	<i>Glomus</i> sp.5	33,3	5.7	19,35	-	-	-
Total		300	100	200	200	100	200

Note: FR = Relative Freq., KR = Relative Abundance and INP = Importance Value Index

Discussion

The results of the study showed that there was no difference in the number of spores based on location. In this study, 36.5 spores were found in L10 and 66.17 in LM10. The average number of spores after trapping was more significant than the number of spores in field soil (Tuheteru et al., 2022). Tuheteru et al. (2022) obtained a spore count of 9/50 g in LM 10 and 2/50g in L 10. Several research results show that trapping can increase the number of spores, including in limestone mines (Suting & Nongthombam, 2021), gold (Tuheteru et al., 2020), and coal (Makdoh and Kayang, 2019). This aligns with Mulyati et al. (2016); the number of spores increased because trapping can stimulate the sporulation of AMF found in soil from the field.

The results of the study showed that there were differences in the number of spores based on the host plant. *Pueraria javanica* plants produced the highest number of spores compared to *Sorghum bicolor*. It is suspected that *P. javanica* has a suitable host in both locations. This is because *P. javanica* (1) has a short life cycle, (2) has High colonization of various AMF, (3) has an intensive root system, and (4) can grow in low P conditions. In addition, *P. javanica* can produce root exudates as a suitable carbon source for producing AMF. This is in line with Brundrett et al. (1996). The selection of host plants that propagate AMF in Culture pots can affect fungal sporulation and root colonization formation and produce inoculum quality. One of the criteria for host selection is an intensive root system. Several studies have shown that *P. javanica* hosts are generally used for AMF propagation (Prinhantoro et al., 2017). And (Daru et al., 2013),



In addition to the number of spores, the effectiveness of AMF on host plants is also characterized by the ability of AMF to colonize the roots of host plants. Chalimah et al. (2007) explained that root colonization is an initial form of the symbiotic process between AMF and the roots of host plants. AMF colonization will play a positive role in providing nutrients and water. Based on the results of the study, it was shown that the host plant *S. bicolor* produced the highest colonization percentage of 49.44% compared to *P. javanica*, but *S. bicolor* had a lower number of spores than *P. javanica*. This aligns with Tuheteru's opinion (2003) that AMF colonization and spores are not significantly correlated with root infection, and the number of spores produced does not necessarily mean a high percentage of root infection. This is also in line with research (Smith & Read, 2008), which explains that differences in colonization percentages are due to differences in type and level of compatibility between AMF and the plant root system. The ability of AMF to colonize roots will influence the growth of the host plant.

In this study, there was also an increase in the number of species found in previous studies; 3 species were found in L10 and two species in LM10 (Tuheteru et al., 2022). New AMFs found in this study were *Acaulosporasp* in LM10 and *Glomus rubiforme* in L10. This is in line with research (Simamora et al., 2015). showed an increase in the number of AMF species from trapping results compared to the number of AMF species found in the field. This is because not all AMF are active in the same period. Some AMFs are abundant in the rainy season, others in the dry season, and others throughout the year. In this study, the most dominant type of AMF was the genus *Glomus*. *Glomus* has a wide host range. This is in line with research (Tuheteru et al., 2022). *Glomus* is a genus that is tolerant to and adaptive to various soil conditions and terrestrial environments. *Glomus* can also survive in acidic to wet soils.

CONCLUSION

Based on the research results, it was concluded that the interaction of *Pueraria javanica* host plants produced an average number of spores of 83.67 spores. The *Glomeraceae* family has the most dominant type of AMF found.

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