



Production of Arbuscular Mycorrhizal Spores by Providing Nasa Liquid Organic Fertilizer (LOF)

Husna^{1*}, Faisal Danu Tuheteru², Asrianti Arif³, Sijebir⁴, Albasri⁵ Wiwin Rahmawati Nurdin⁶, Parwito⁷, Mariana Zainun⁸

^{1,2,3,4,5,6,8}Department of Forestry, Faculty of Forestry and Environmental Sciences, Halu Oleo University, Kendari, Southeast Sulawesi. 93121, Indonesia

⁷Department of Agrotechnology, Faculty of Agriculture, Universitas Ratu Samban, Bengkulu, 38611, Indonesia

*Corresponding author : husna@uho.ac.id

ABSTRACT. Arbuscular mycorrhizal fungi (AMF) are root symbionts that are in symbiosis with the majority of plants and are generally found in terrestrial ecosystems. The propagation of AMF is influenced by many factors, including fertilization. This study aims to determine the increase in arbuscular mycorrhizal spore production by administering Nasa LOF. The research was conducted at the Plastic House of the Indonesian Mycorrhizal Association, Southeast Sulawesi Branch and the Laboratory of the Forestry Department, Faculty of Forestry and Environmental Sciences, UHO in January - April 2023 using a factorial Completely Randomized Design (CRD) with two factors, namely the first factor, the type of AMF including *Glomus coronatum* and *Glomus claroideum* and the second factor is Nasa LOF control, 2.5 ml/1000 ml water, 5 ml/1000 ml water. The results showed that the combination treatment of *Glomus claroideum* and administration of 5 ml Nasa LOF increased the number of spores in the 3rd month and spores after drying. Nasa LOF 5 ml is relatively applied for the production of *Glomus claroideum*.

Keywords : *Glomus claroideum*, Nasa LOF, Production spores

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are root symbionts that are in symbiosis with the majority of plants and are generally found in terrestrial ecosystems (Smith and Read, 2008; Rombe et al., 2022). Arbuscular mycorrhizal fungi are a type of fungus that benefits plant growth, especially in soils that experience a lack of phosphorus (Rivana et al., 2019). Arbuscular mycorrhizal fungi (AMF) are also known as biofertilizers that can increase soil and plant productivity (Nurbaity et al., 2009). Apart from that, mycorrhiza is an effort to overcome stunted growth due to drought stress (Muis et al., 2013). Based on the potential importance of AMF for soil and plant quality, it can be developed as a biological fertilizer by producing (propagating) mycorrhizal spores..

One way to propagate arbuscular mycorrhizal fungi (AMF) is by producing AMF inoculant which is generally relatively simple, namely by using inorganic carrier materials such as sand or zeolite. The most important thing in this production process is the availability of human resources, good quality starter inoculum as well as host plants and production facilities (Nurbaity et al., 2009). According to Muis et al., (2013); Rungkat (2009), the way mycorrhiza works is by infecting the root system of the host plant and producing a network of hyphae so that plants containing mycorrhiza will be able to increase their capacity to absorb nutrients. According to Brundrett et al., (1996) mycorrhizal fungi can be propagated or cultured using pot culture techniques using certain host plants. Host plants that are often used are *Peuraria javanica* (pj), a type of legume cover crop (LCC), sorghum, corn and soybeans. However, different types of host plants and inoculum sources will influence the infectivity

and effectiveness of mycorrhizal production (Nurhayati, 2019). Apart from that, fertilization factors also influence mycorrhizal production (Indriani et al., 2019). Therefore, it is necessary to fertilize to support the quality of AMF spores and colonization (Uswatun, 2017).

Fertilizer is a producer of the main nutrients which play a role in determining the level of growth and production of plants, because fertilizer contains the raw materials that plants need to grow. Meanwhile, fertilization can be interpreted as providing organic or non-organic materials to replace nutrient losses in the soil and to meet the nutrient needs of plants so that plant productivity increases (Mansyur et al., 2019). Nasa's liquid organic fertilizer (LOF) is a 100% natural organic fertilizer from extracts of organic material from livestock and poultry waste, certain plant waste and other natural substances which are processed based on environmentally sound technology. This is in line with research (Husna et al., 2017) which uses red hyponex fertilizer as a source of nutrition for plants. Nasa LOF is able to accelerate plant growth, can reduce the level of pest attacks, has no side effects that are detrimental to plants and the environment, and is safe for humans (Nugrahini, 2013). Therefore, it is important to carry out this research to find out how Nasa LOF influences the production of Arbuscular Mycorrhizal Fungi (AMF).

MATERIALS AND METHODS

Location and Time of Research. This research was carried out at the Screen House of the Indonesian Mycorrhizal Association (AMI) Southeast Sulawesi Branch and the Forestry Laboratory unit of the Faculty of Forestry and Environmental Sciences, Halu Oleo University, Kendari, which lasted for 4 months from January to May 2023.

Research Design. This research was structured based on a Completely Randomized Factorial Design, which consists of 2 factors. First factor: Type of AMF which consists of 2 levels, namely *Glomus coronatum* (A1) and *Glomus Claroideum* (A2). Second factor: Fertilizer application consisting of 3 levels, namely without fertilizer (B0), 2.5 ml fertilizer/pot (B1), 5 ml/pot fertilizer (B2) with 3 repetitions. So there are 18 treatment combinations. Each treatment unit contained 9 plants so that the total of all plants was 162 plants.

Research procedure.

1. Preparation of growing media

Washed thoroughly to remove fine zeolite powder and existing dirt. Unclean zeolite rock can have a negative impact on the development of AMF. Then sterilize with a sterilizer for 8 hours to eliminate possible pathogens. After that, the zeolite rock was soaked in Nasa LOF solution at a dose of 1 ml/liter of water for 24 hours (Nurrobifahmi et al., 2017).

2. Preparation of plant host

The *Pueraria javanica* used as a host plant is first soaked in bayclin solution at a dose of 2 ml/1 liter of water for 5 minutes, then rinsed with clean water 3 times. The seeds are soaked in hot water at 50 degrees for \pm 24 hours to break any dormancy that may occur. After that, the seeds can be planted directly in culture pots.

3. AMF propagation

The propagation of AMF in this study used zeolite media. The media is filled in the container provided with a ratio of 150 g of zeolite and 5 grams of inoculum containing AMF.

4. Maintenance



Culture maintenance activities include watering twice a day with 30 ml per culture pot. Every 2 (two) weeks fertilization is carried out with Nasa LOF fertilizer. Apart from that, manual weeding and pest control are carried out.

Variables. Variables measured in this study included number of spores, root colonization, number of leaves, number of nodules, and plant dry weight (shoots, nodules, and roots).

Data Analysis. The data analysis used was the analysis of variance (F test) and continued with the different treatment tests according to Duncan Multiple Range Test (DMRT) at a 95% confidence level using the SAS version 9.1.3.

RESULTS AND DISCUSSION

A recapitulation of the results of variance (F test) of the treatment of Arbuscular Mycorrhizal Fungi (AMF) and the application of Nasa LOF is presented in table 1. Table 1 shows that the combination of AMF and Nasa LOF Fertilizer (A*B) has a very significant effect on AMF colonization in the month 1st, 2nd and the number of nodules and shoot dry weight, and had a significant effect on AMF spores in the 2nd, 3rd months and spores after drying, AMF colonization in the 3rd month, and root dry weight. Except for 1st month AMF spores and number of leaves.

Table 1. Recapitulation of results of analysis of variance of the effect of AMF species and Nasa LOF treatment on spores number and AMF colonization.

Variables	Treatment			CV
	AMF species (A)	Nasa LOF (B)	A*B	
AMF spore Month 1st	*	**	ns	19.64
AMF spore Month 2nd	ns	**	*	14.62
AMF spore Month 3rd	**	**	*	17.33
AMF spore after drought	**	**	*	11.71
Root colonization Month 1st	**	ns	**	16.83
Root colonization Month 2nd	ns	ns	**	14.11
Root colonization Month 3rd	**	**	*	8.01
Leaf number	**	**	ns	4.79
Nodul dry weight	*	**	**	2.38
Shoot dry weight	ns	ns	**	3.20
Root dry weight	tn	tn	*	2.73

Description: ** = Very significant effect, ns = No significant effect, * = Significantly effect, CV = Coefficient of variant.

The results of the Duncan test on the effect of the combination of AMF treatment and Nasa LOF fertilizer on the number of spores are presented in Table 2. Table 2 shows that the combination treatment of *G. coronatum* with 5 ml of fertilizer produced the most spores in the second month with 32 spores and was not significantly different. with other treatments except the *G. claroideum* treatment without fertilizer and the *G. coronatum* treatment without fertilizer. Treatment of the *G. claroideum* AMF type by applying 5 ml of fertilizer. In the 3rd month and after drying, it produced the most spores and was significantly different from other treatments.

Tabel 2. The effect of AMF species and Nasa LOF on AMF spores

Treatment		AMF spores		
AMF	POC Nasa (ml)	Month 2nd	Month 3rd	After drought
<i>Glomus</i>	Control	13.67c	47.00c	87.00c
<i>Coronatum</i>	2.5	26.67ab	61.00c	101.00c
	5	32.00a	72.00c	112.00c
<i>Glomus</i>	Control	21.66b	60.33c	100.33c
<i>Claroideum</i>	2.5	27.33ab	115.67b	155.67b
	5	26.33ab	143.67a	183.67a
Pr > F		0.0210	0.0123	0.0123

Note: The average value followed by the same letter in the same column is not significantly different at the DMRT test level at the 95% confidence level.

The results of the Duncan Test on the effect of the combination of AMF treatment and Nasa LOF fertilizer on root colonization are presented in Table 3. Table 3 shows that the *G. Claroideum* without fertilizer resulted in the most colonization in the 1st month and was significantly different from the other treatments. The *G. claroideum* without fertilizer in the second month produced the highest colonization of 42.50 and was not significantly different from the other treatments. except for the *G. coronatum* without fertilizer and *G. claroideum* with 5 ml of fertilizer. Treatment of the *G. claroideum* with 5 ml of fertilizer resulted in the highest colonization in the 3rd month of 61.66 and was not significantly different from other treatments. except for the *G. coronatum* treatment with 5 ml, 2.5 ml fertilizer and without fertilizer.

Table 3. Effect of the combination of AMF and Nasa LOF on root colonization

Treatment		MAF Colonization (%)		
AMF	Nasa LOF (ml)	Month 1st	Month 2nd	Month 3rd
<i>Glomus</i>	control	12.50c	25.83c	37.50c
<i>Coronatum</i>	2.5	19.16b	36.66ab	52.50b
	5	15.83bc	33.33abc	51.66b
<i>Glomus</i>	control	25.00a	42.50a	56.66ab
<i>Claroideum</i>	2.5	17.50bc	35.16ab	58.33ab
	5	19.16b	35.50bc	61.66a
Pr > F		0.0056	0.0085	0.0506

Note: The average value followed by the same letter in the same column is not significantly different at the DMRT test level at the 95% confidence level.

The results of the duncan test of the combination of AMF and Nasa LOF treatments on the dry weight of shoots, roots and dry weight of nodules are presented in Table 4. Table 4 shows that the *Glomus coronatum* with 5 ml of fertilizer produced the highest dry weight of shoots with a value of 6.81g and not significantly different from other treatments. except for the treatment of *G. claroideum* with 5 ml fertilizer and *G. coronatum* without fertilizer. The *G. claroideum* AMF type without fertilizer produced the highest dry root weight with a value of 6.07g and was not significantly different from other treatments. except for AMF *G. coronatum* treatment without fertilizer. In the *G. claroideum*, 2.5 ml of fertilizer was applied to produce the highest dry weight of nodules, namely 2.56 g and was significantly different from other treatments.

Table 4. The effect of treatment of FMA and NASA LOF on plant dry weight

Treatment		Plant Dry weight (g)		
AMF	Nasa LOF (ml)	Pucuk	Akar	Nodul
<i>Glomus</i>	Kontrol	6.07c	5.68b	2.23c
<i>Coronatum</i>	2.5	6.49ab	5.80ab	2.35b
	5	6.81a	6.01a	2.32b
<i>Glomus</i>	Kontrol	6.71a	6.07a	2.29bc
<i>Claroideum</i>	2.5	6.64ab	6.06a	2.56a
	5	6.37bc	5.89ab	2,31bc
Pr > F		0.0028	0.0478	0.0057

Note: The average value followed by the same letter in the same column is not significantly different at the DMRT test level at the 95% confidence level.

The results of the Duncan test on the independent effect of AMF treatment on the number of spores in the 1st month and the number of leaves are presented in Table 5. Table 5 shows that the *G. claroideum* treatment in the 1st month can increase the number of spores by 6.66 spores and is significantly different from the *Glomus coronatum* treatment. The *G. claroideum* increased the number of leaves by 35.33 pieces and was significantly different from the *G. coronatum* treatment.

Table 5. The effect of AMF on spore number and leaf number

AMF	Spore number month 1st	Leaf number
<i>Glomus coronatum</i>	5.33b	32.89b
<i>Glomus claroideum</i>	6.66a	35.33a
Pr>f	0.0335	0.0080

Note: The average value followed by the same letter in the same column is not significantly different at the DMRT test level at the 95% confidence level.

The results of the Duncan test on the effect of Nasa LOF treatment on the number of spores in the first month and the number of leaves are presented in Table 6. Table 6 shows that the treatment of giving 5 ml Nasa LOF produced the highest number of spores and number of leaves and was significantly different from other treatments.

Tabel 6. The effect of Nasa LOF on spore number and leaf number

Nasa LOF	Spore number month 1st	Leaf number
B0	3.50c	30.00c
B1	6.33b	35.00b
B2	8.16a	37.33a
Pr > F	<.0001	<.0001

Note: The average value followed by the same letter in the same column is not significantly different at the DMRT test level at the 95% confidence level.

The results of the study showed that there were differences in the combined effect of AMF type treatment and Nasa LOF dosage on AMF colonization and sporulation. The number of AMF spores and colonization continued to increase from month 1 to month 3 until the spores were dry. *Glomus claroideum* AMF treatment produced more sporulation compared to *glomus coronatum*. This is because AMF types have different characteristics or

abilities in responding to the fertilizer provided and the glomus type AMF is classified as a type that is sensitive to fertilizer application (Husna et al., 2017). This is in line with previous research which stated that spores of the Glomus species were reported to grow well on all types of nutrition (Delvian, 2008).

The combination of *Glomus claroideum* treatment with 5 ml Nasa LOF resulted in the highest number of spores after drying at 183.67 spores. *Glomus claroideum* with 5 ml of fertilizer has the ability to adapt and is able to produce spores. This shows that the application of fertilizer greatly influences AMF production (Hasanah et al., 2017).

Apart from the number of spores, the effectiveness of AMF on host plants is also characterized by AMF's ability to colonize the roots of the host plant. Chalimah et al. (2007) explained that root colonization is the initial form of a symbiotic process between AMF and host plant roots. AMF colonization will provide a positive role in providing nutrients and water. Based on the research results, it shows that the *Glomus claroideum* type given 5 ml Nasa LOF produced the highest colonization percentage of 61.66 compared to other types of treatment. This shows that the *Glomus claroideum* type has a high level of suitability for the host plant and shows that each treatment has a different level of ability to colonize the roots of the pueraria plant. This is in line with the opinion of Smith and Read (2008), who explained that the difference in colonization percentage was due to differences in the type and level of compatibility between AMF and the plant root system. AMF's ability to colonize roots will have an influence on the growth of the host plant. Musafa et al. (2015) explained that the higher the AMF colonization on plant roots, it can be indicated that the mycorrhizal spores are more active in infecting the roots and expanding the root absorption area for water and nutrients, so that plants can grow optimally.

Based on the results of research conducted on the treatment of *Glomus coronatum* with 5 ml Nasa POC and *Glomus claroideum* with 2.5 ml Nasa POC were able to increase the dry weight of shoots, roots and nodules. This is because the glomus has larger intraradical hyphae (Dodd et al., 2000). Intraradical hyphae are able to transfer metabolic nutrients and water from outside to the root cortex of the host plant, while less extensive extraradical hyphae result in less carbon flow to the rhizosphere in colonized plants (Souza, 2015).

In general, the results of research on the independent influence of 5 ml Nasa LOF were able to increase spores in the 1st month and the number of leaves with the highest yields of 8.16 and 37.33 respectively. This shows that giving Nasa POC in a dose of 5 ml has a very good level of compatibility with *Puraria javanica*, which is thought to be influenced by the ability of mycorrhizal roots to absorb nutrients and water. Root development is due to the presence of AMF which increases root interception in absorbing nutrients and water (Rengganis. 2013).

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that treatment of *Glomus claroideum* and 5 ml Nasa liquid organic fertilizer (LOF) can provide the best results for increasing the number of spores in the 3rd month and after drying. 2. Independent treatment of AMF and Nasa LOF was able to increase the number of 1st Month Spores and the number of leaves.



REFERENCES

- Chalimah, S., Muhadiono., L. Aznam., S. Haran dan N. T. Mathius. 2007. Perbanyak Gigaspora Sp. dan Acaulospora Sp. dengan Kultur Pot di Rumah Kaca. *Jurnal Biodeversitas*. 7 (4):12-19
- Delvian. 2008. Pengaruh spesies inang dan sumber nutrisi terhadap produksi spora fungi mikoriza arbuskula. *Jurnal Nature Indonesi*. 10 (2): 70-72.
- Dodd, J. C., C. L. Boddington, A. Rodriguez, C. Gonzalez Chavez, and I. Mansur. 2000. Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant Soil*. (226): 131–151.
- Hasanah, S. U., Husna., F. D. Tuheteru., A. Arif., Basrudin dan W. R. Nurdin. 2017. Sporulasi Fungi Mikoriza Arbuskula Lokal Asal Rizosfer Kayu Kuku (*Pericopsis Mooniana* [Thw] Thw.) Dengan Pemberian Takaran Terabuster Yang Berbeda. *Prosiding seminar nasional mikoriza*. 191-194
- Husna., F. D. Tuheteru., A. Arif dan A. F. Renggaala. 2017. Sporulasi fungi mikoriza arbuskula lokal asal rizosfer kayu kuku [*Pericopsis mooniana* (thw.) Thw] yang dipengaruhi takaran hyponex merah. *Ecogreen*. 3(2): 79- 87.
- Mansyur, N. I., E. H. Pudjiwati dan A. Murtalaksono. 2019. Pupuk dan Pemupukan. Syiah Kuala University Press dan Universitas Borneo Tarakan.
- Muis, A., D. Indradewa dan J. Widada. 2013. Pengaruh Inokulasi Mikoriza Arbuskula Terhadap Pertumbuhan dan Hasil Kedelai (*Glycine Max* (L.) Merrill) Pada Berbagai Interval Penyiraman. *Vegetalika*. 2(2):7-20.
- Musafa, M.k., L.Q, Ain dan B, Prasetya. 2015. Peran mikoriza arbuskula dan bakteri *Pseudomonas fluorescens* dalam meningkatkan serapan P dan pertumbuhan tanaman jagung pada andisol. *Jurnal Tanah dan Sumberdaya Lahan*. Vol. 2(2):191-197.
- Nugrahini, T. 2013. Respon Tanaman Bawang Merah (*Allium Ascolonicum* L.) Varietas Tuk Tuk Terhadap Pengaturan Jarak Tanam dan Konsentrasi Pupuk Organik Cair Nasa. *Ziraa'ah*. 36(1):60-65.
- Nurbaity, A., D. Herdiyantoro dan O. Mulyani. 2009. Pemanfaatan Bahan Organik Sebagai Bahan Pembawa Inokulan Fungi Mikoriza Arbuskula. *Jurnal Biologi*. 13(1):17-11.
- Nurrobifahmi., I. Anas., Y. Setiadi dan Ishak. 2017. Pengaruh Metode Sterilisasi Radiasi Sinar Gamma Co-60 dan Autoklaf Terhadap Bahan Pembawa, Viabilitas Spora *Gigaspora Margarita* dan Ketersediaan Fe, Mn, Dan Zn. *Jurnal Tanah Dan Iklim*. 41 (1): 1-8.
- Rengganis, D. 2013. Studi Keanekaragaman Genus Fungi Mikoriza Arbuskula di Sekitar Perakaran Pohon Jabon (*Anthocephalus cadamba* Robx Miq.) Alami [skripsi]. IPB. Bogor
- Rivana, E., N.P. Indriani dan L. Khairani. 2019. Pengaruh Pemupukan Fosfor dan Inokulasi Fungi Mikoriza Arbuskula (FMA) Terhadap Pertumbuhan dan Produksi Tanaman Sorghum (*Sorghum Bicolor* L.). *Fakultas Peternakan*.
- Rombe, Y., Husna., F. D. Tuheteru dan A. Arif. 2022. Production Of Arbuscular Mycorrhizal Fungi Spores (AMF) *Glomus Coronatum* and *Glomus Claroideum* With Ab Mix Nutrition. *Journal Of Tropical Mycorrhiza*. 1(2):87-88.

Smith, S.E dan Read D.J. 2008. Mycorrhizal symbiosis. Third ed. New York (US): Academic Press.
Souza, T. 2015. Handbook of Arbuscular Mycorrhizal Fungi. Springer.