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Production of Arbuscular Mycorrhizal Fungi Spores (AMF) Glomus Coronatum and Glomus Claroideum With Ab Mix Nutrition

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ABSTRACT. Arbuscular Mycorrhizal Fungi (AMF) is an obligate fungus that has a symbiosis with plant roots and can stimulate plant growth and productivity so that AMF has the potential to be developed as a biological fertilizer. AMF propagation is influenced by many factors including fertilization. This study aims to determine the effect of giving AB mix nutritional doses on the production of AMF types of Glomus coronatum and Glomus claroideum. The research was conducted at the Plastic House of the Indonesian Mycorrhizal Association Southeast Sulawesi Branch and the Laboratory of the Department of Forestry, Faculty of Forestry and Environmental Sciences, UHO in September - December 2022 using a factorial Completely Randomized Design (CRD) with two factors, namely the first factor was AMF type including Glomus coronatum and Glomus claroideum and the second factor is AB mix nutrition including control, 2.5 ml/1000 ml water, 5 ml/1000 ml water. The results showed that the interaction treatment of AMF Glomus claroideum and the provision of 2.5 ml AB mix nutrition increased the number of AMF spores by 35 spores/5 g zeolite and root colonization by 69.16%.

Keywords: *Glomus claroideum, AB nutrition mix,Spore production.*

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) is a root symbiont that forms a symbiosis with the majority of plants and is commonly found in terrestrial ecosystems (Smith and Read, 2008).Arbuscular mycorrhizal fungi are obligate fungi of the phylum Glomeromycota, the endomycorrhizal group, in which the fungal hyphae enter plant cells which form tall branched structures called arbuscules (Husna et al., 2018; Kobayashi et al., 2018), which serve as exchange sites nutrition between fungi and plants (Selvakumar et al., 2018). AMF can be associated with almost 90% of plant species(Smith and Read, 2008).

Arbuscular mycorrhizal fungi have an important role in the forestry sector. AMF is reported to increase the success of land and forest restoration (Wanget al., 2019), rescue of endangered species (Husna et al., 2018), and mine reclamation (Asmarahman et al., 2018; Husna et al., 2021). AMF is involved in increasing plant tolerance to biotic and abiotic stresses (Husna et al., 2016; Alotaibi et al., 2021), increasing the rate of photosynthesis (Rini et al., 2020; Yuwati et al., 2021; Husna et al., 2017), and as a biological fertilizer (Sari and Indrawati, 2019).

Based on the above roles, AMF has the potential to be developed as a biological fertilizer. An important stage in the development of AMF is the production of AMF spores. The production of AMF spores can be carried out using a simple method, namely using pot culture (Ghosh and Dutta, 2018), using one or more types of AMF (Nusantara et al., 2010). Brundrett et al., (1996) described that in AMF propagation efforts the level of effectiveness and spore multiplication was greatly influenced by several factors, namely the type of host plant, nutrients, and media. In addition, the fertilization factor also has a significant effect on the production of AMF spores (Indriani et al., 2019). This is in line with research (Husna et al., 2017) which used red hyponex fertilizer as a source of nutrition for plants.

One source of nutrition that can be used is AB mix nutrition. AB mix nutrition is a fertilizer that comes from salts and minerals dissolved in water (Setiawan, 2018). AB mix nutrition is divided into two stocks, namely stock A and stock B. Stock A contains compounds containing Ca, while stock B contains compounds containing sulfates and phosphates. 2016). AB mix nutrition has 16 essential elements needed by plants, namely macro nutrients (N, P, K, Ca, Mg, S), and micro nutrients (Fe, Mn, Cu, Zn, Bo, Mo).which is 100% water soluble, so it is easily absorbed and can meet plant nutrition. At present, propagation of arbuscular mycorrhizal fungi has been carried out, but AMF propagation, especially the glomus species using AB mix nutrition, has never been carried out. Therefore, it is important to conduct research on the production of arbuscular mycorrhizal fungi spores by administering AB mix nutrition. In order to determine the best interaction effect on AMF spore production and to determine the effect of AMF type treatment and/or AB mix nutrition on AMF spore production.

MATERIAL AND METHODS

Location and Time of Research.

This research will be carried out at the Plastic House of the Indonesian Mycorrhiza Association (AMI) Southeast Sulawesi Branch and the Laboratory of the Department of Forestry, Faculty of Forestry and Environmental Sciences (FHIL) Halu Oleo University (UHO) at position 3º57'56,3S 122º31'52.6 E which lasts for 3 months.

Research Design.

This study was designed using a completely randomized design (CRD) which was arranged factorially. The factorial design pattern is composed of 2 factors: AMF type factor (A) consists of A1: Glomus coronatum, A2: Glomus claroideum and Fertilization Factor (B) consists of B0: Control, B1: Treatment with 2.5 ml of AB nutrient mix/1000 ml of water, B2: Treatment with 5 ml of nutrients AB mix / 1000 ml of water.

Research Procedure.

1. Culture Media Preparation

a) The petri dish that will be used as a place for planting culture must first be perforated at the edges which serves as a place for the emergence of plants.
b) The petri dish is filled with zeolite until it is full and sufficiently dense before the zeolite is sterilized using an autoclave.with a temperature of 121oC to prevent the incorporation of pathogens or nematodes that can damage the culture and zeolite immersed in a nutrient solution AB mix at a dose of 2.5 ml/liter of water for 24 hours.

2. Germination of Pueraria javanica Seeds

a) P. javanica seeds to be used as host plants were first soaked in bayclin solution for 5-10 minutes as an effort to surface sterilization.
b) Then soaked in warm water for ± 24 hours to break dormancy that might occur.
c) The seeds were sown in germination tanks ± 10 days. After that, you can start planting immediately.

3. Culture Creation

a) P. javanica sprouts that already have 2-3 leaves (7-10 days after sowing) are placed on white paper or tissue.

Journal of Tropical Mycorrhiza
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Vol. 1 No. 2 October 2022

b) Spores isolated from trapping that had been collected in a watch glass were taken with spore tweezers and placed on the roots of the P. javanica seedlings. Each seedling was inoculated with only one spore.
c) The inoculated P. javanica sprouts were then carefully transferred to the culture medium with the stem of the seedling placed on the edge of the perforated petri dish.
d) Furthermore, the petri dish is closed with a lid and given adhesive (tape) at four points to prevent the culture from spilling. Then each cultured petri dish was labeled containing data on the date the culture was made, the plot number from which the culture was made (indicating the location point in the field), the type of spore inoculated or cultured and the maker of the culture.
e) Furthermore, the culture of the petri dish was wrapped in aluminum foil to reduce the direct influence of light on the culture medium. The petri dish culture was then placed in a plastic tub which had been wrapped in black plastic which served as a container for water and nutrient solution for the culture. Giving water through a plastic tub is done according to the needs of the plants.
f) Provision of AB Mix nutritional fertilizer at various levels of concentration doses (0, 2.5 ml, and 5 ml) where AB Mix nutrition is carried out once every 2 weeks
g) The culture will be maintained for 3 months to find out the progress of the sporulation process, so the culture will be observed every month starting in the first month after making the culture.

Variable

1. Dry Weight

a) From each plant, shoots, roots and nodules were taken, then these parts were put into a thick envelope.
b) Furthermore, the envelopes of shoots, roots and nodules were baked in the oven at 70°C for 2 x 24 hours.
c) Then do the weighing.

2. Number of AMF Spores

From the results of germination and sporulation of single spores, the number of spores produced at the end of the observation can be calculated using the pour filter method (Pacioni, 1992; Husna et al., 2014). The filter pouring technique procedure is carried out as follows:

a) Take 5 grams of zeolite samples. Then filtered in a set of filters with sizes of 710 µm, 125 µm, and 45 µm sequentially from top to bottom. From the top of the filter, it is sprayed with tap water to make it easier for the spores to escape. Then the top filter is removed from the second filter and then sprayed again with tap water.
b) After the second filter is removed, the last filtered water is poured into the petri dish.
c) then observed and counted the number of spores under a binocular microscope and written down the results.

3. AMF Colonization on Sample Plant Roots (%)

AMF colonization was observed from month 1 to month 3 through root staining techniques. Anatomical characteristics that characterize the presence or absence of AMF infection cannot be seen directly, unless the roots of the sample are stained and viewed under a

microscope. Therefore staining of sample roots is very important in observing and identifying AMF infection in host plant roots (Brundrett et al., 1996 in Husna et al., 2015).

a) Select fine roots by opening the petridish and taking 15 cm fresh roots then washing them with running water until clean. Root samples were soaked in 10% KOH solution until the roots became clear in color ± 24 hours. If the root contains a lot of phenol, the solution will be dark brown.
b) The KOH solution was then discarded and the root samples were washed in running water for 5-10 minutes.
c) The root samples were then immersed in 2% HCl solution for 30 minutes, and in this process the roots would turn white or pale. The 2% HCl solution was then removed by flowing it slowly.
d) Furthermore, the root samples were soaked in 0.05% Trypan blue solution for ± 24 hours.
e) Then the 0.05% Trypan blue solution was discarded and replaced with glycerol solution for ± 24 hours.

Furthermore, observation activities to determine the percentage of AMF colonization in root samples were ready to be carried out under a microscope. 10 pieces of root that had been stained with a length of ± 1 cm were randomly taken and arranged on glass slides. Root colonization is indicated by the presence of hyphae, vesicles, arbuscles or one of the three. Each field of view of the microscope that shows signs of colonization is marked with a (+) symbol and if not colonized, it is marked with (-). The percentage of root colonization was calculated using the formula:

$$\text{Colonized root (\%)} = \frac{\sum \text{colonized field of view}}{\sum \text{total field of view}} \times 100\%$$

Data Analysis.

Observational data were analyzed using analysis of variance (F test). If the test results show a significant effect, a different treatment test will be carried out according to the Duncan Multiple Range Test (DMRT) at a 95% confidence level. Data analysis using portable SAS 9.0 software.

RESULT AND DISCUSSION

Recapitulation of the results of the analysis of variance (F test) for the types of Arbuscular Mycorrhizal Fungi (AMF) and AB mix nutrition for the observed variables is presented in Table 1. Table 1 shows that the interaction of AMF types and AB mix nutrient doses had a very significant effect on root dry weight and nodule dry weight, and had a significant effect on the number of spores (8 wp-12 wp) and after drying), AMF colonization (8 wp-12 wp), and shoot dry weight but had no significant effect with the observed variables spore number 4 wp, AMF colonization 4 wp, total dry weight and number of leaves. Independently, the type of AMF (A) had a very significant effect on the number of spores (4 wp-12 wp and after drying), AMF colonization (4 wp-12 wp), and root dry weight and had a significant effect on the number of leaves, total dry weight and dry nodules but had no effect on shoot dry weight.

The effect of independent factors on the AB mix nutrient dosage treatment had a very significant effect on AMF colonization (8 wp and 12 wp), root dry weight, total dry weight, nodule dry weight and number of leaves and had a significant effect on spore count (8 wp-12 wp and after drying) and shoot dry weight. However, the nutritional effect of AB mix had no significant effect on other variables.

Journal of Tropical Mycorrhiza
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Vol. 1 No. 2 October 2022

Table 1. Recapitulation of the various effects of AMF and the application of AB mix nutrition on research variables

Variable	A*B	AMF (A)	Fertilizer (B)	CV
Spore Count 4 wap	mr	*	mr	24.00
Spore Count 8 wap	*	**	*	21.24
Spore Count 12 wap	*	**	*	11.85
Number of Spores After Drying (14 wap)	*	**	*	12.35
AMF colonization 4 wap	mr	**	mr	22.41
AMF colonization 8 wap	*	**	**	10.32
AMF colonization 12 wap	*	**	**	10.12
Shoot Dry Weight	*	mr	*	16.83
Root Dry Weight	**	**	**	5.46
Total Dry Weight	mr	*	**	10.25
Nodule Dry Weight	**	*	**	2.37
Number of Leaves	mr	*	**	9.01

Note: (P≤0.01) = Very significant impact (**) , (P≤0.05) = Significantly influential (*) , (P≥0.01) and (P≥0.05) = Not significant real (tn). (A) : Mycorrhiza/AMF, (B) : AB Mix Nutrition. Coefficient of Diversity: (CV).

Effect of Interaction of AMF and AB Mix Nutrition Dosage

The results of the Duncan test, the effect of the interaction between AMF type treatment and AB mix nutrition on the number of spores is presented in Table 2. Table 2 shows that the *G. claroideum* type AMF treatment without AB mix nutrition produced the most spores at 8 wap as many as 4 spores and were not significantly different from the other treatments except with the treatment of AMF *G. coronatum* without AB mix nutrition. In the 3rd month, the treatment of AMF G. claroideum without AB mix nutrition was significantly different from other treatments except for the treatment of AMF *G. claroideum* with 5 ml of AB mix nutrition. The interaction of AMF and AB nutrition mix after drying produced the most spores on AMF *G. claroideum* with 2.5 ml of AB mix nutrition of 35 spores/5 grams and significantly different from other treatments except for AMF *G. Claroideum.*

Table 2. Interaction of AMF Types and AB Mix Nutrition on Spore Counts

Treatment		Number of Spores/ 5 g of zeolite		
AMF Type (A)	AB Mix (B)	8 wap	12 wap	After Drying
A1	B0	1b	11bc	18d
	B1	3a	10c	28bc
	B2	3a	10c	25c
A2	B0	4a	19a	34ab
	B1	3a	13b	35a
	B2	3a	18a	29bc
Pr>F		0.0029	<.0001	0.0006

Note: The mean value of the variable and the reference value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at 95% confidence. B0 (control), B1 (AB nutrition mix 2.5 ml/ 1000 ml water), B2 (AB nutrition mix 5 ml/ 1000 ml water) A1 (*G. coronatum*), A2 (*G. claroideum*).

The results of the duncan test on the interaction of AMF type treatment and AB mix nutrition on colonization are presented in Table 3. Table 3 shows that AMF colonization at 8

wap and 12 wap was highest in the *G. claroideum* treatment with 2.5 ml AB mix nutrition giving successive results - 54.15% and 69.16% respectively and not significantly different from the type of AMF *G. coronatum* treatment with 2.5 ml of AB mix nutrition, and the type treatment of AMF *G. claroideum* without AB mix nutrition and significantly different to the type of AMF *G. coronatum* without AB mix nutrition, AMF *G. coronatum* type treatment with 5 ml AB mix nutrition and AMF *G. claroideum* type treatment with 5 ml AB mix nutrition.

Table 3. Interaction of AMF Types and AB Mix Nutrition on AMF Colonization

Treatment		AMF colonization (%)	
AMF Type (A)	**AB Mix (B)**	**8 wap**	**12 wap**
A1	B0	32.50d	46.66b
	B1	51.66a	59.16a
	B2	40.83c	44.16b
A2	B0	50.00ab	63.33a
	B1	54.16a	69.16a
	B2	42.50bc	43.33b
Pr>F		0.0009	0.0003

Note: The mean value of the variable and the reference value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at 95% confidence. B0 (control), B1 (nutritional AB mix 2.5 ml/1000ml water), B2 (nutritional AB mix 5ml/1000ml water) A1 (*G. coronatum*), A2 (*G. claroideum*).

The results of the Duncan test on the interaction of AMF type and AB mix nutrition on total dry weight of plants (shoots, roots, nodules) are presented in Table 4. Table 4 shows that *G. coronatum* with 2.5 ml of AB mix nutrition produced shoot dry weight the highest was 3.95g with an increase value of 49.06% and significantly different from other treatments except for the type of *G. coronatum* with 2.5 ml of AB mix nutrition and *G. claroideum* type with 5 ml of AB mix nutrition. In the dry weight variable of *G. coronatum* roots with 5 ml of AB mix nutrition gave the highest value of 3.95 with an increase of 58.63% and was significantly different from other treatments.

The interaction of AMF treatment and AB mix nutrition increased the highest nodule dry weight in the AMF *G. claroideum* type treatment with 2.5 ml of AB mix nutrition of 2.56 g with an increase value of 11.79% and significantly different from the AMF *G. coronatum* type treatment without nutrition AB mix, *G. coronatum* treatment with 2.5 ml AB mix nutrition, AMF *G. coronatum* treatment with 5 ml AB mix nutrition, *G. claroideum* treatment without AB mix nutrition and *G. claroideum* treatment with providing nutrition AB mix 2.5 ml.

The interaction of AMF treatment and AB mix nutrition increased the highest nodule dry weight in the *G. claroideum* type treatment with 2.5 ml of AB mix nutrition of 2.56 g with an increase value of 11.79% and significantly different from the *G. coronatum* type treatment without nutrition AB mix, *G. coronatum* treatment with 2.5 ml AB mix nutrition, *G. coronatum* treatment with 5 ml AB mix nutrition, *G. claroideum* treatment without AB mix nutrition and *G. claroideum* treatment with providing nutrition AB mix 2.5 ml.

Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 1 No. 2 October 2022

Table 4. Interaction of Types of AMF and AB Mix Nutrition on Dry Weight

Treatment		Plant Dry Weight (g)					
AMF Type (A)	AB Mix (B)	Shoots	% enhancement	Root	% enhancement	nodule	% enhancement
A1	B0	2.65b	-	2.49c	-	2.23c	-
	B1	3.95a	49.06	2.81b	12.85	2.35b	5.38
	B2	3.25ab	22.64	3.95a	58.63	2.36b	5.83
A2	B0	2.45b	-	2.34c	-	2.29bc	-
	B1	2.65b	8.16	3.01b	28.63	2.56a	11.79
	B2	3.63a	48.16	3.10b	32.48	2.31bc	0.87
Pr>F		0.0177		<.0001		0.0003	

Note: The mean value of the variable and the reference value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at 95% confidence. B0 (control), B1 (nutritional AB mix 2.5 ml/1000ml water), B2 (nutritional AB mix 5ml/1000ml water) A1 (*G. coronatum*), A2 (*G. claroideum*).

Influence of Independent AMF (A)

The results of the duncan test for the effect of AMF treatment on the number of spores at 4 wap, colonization at 4 wap, total dry weight and number of leaves are presented in Table 5. Table 5 shows that AMF treatment with *G. claroideum* at 4 wap increased the number of spores by 1 spore and did not differ significantly with the *G. coronatum* treatment, increased AMF colonization by 27.50% at 4 wap, and significantly different from the *G. coronatum* treatment, and increased the number of leaves by 45 strands and significantly different from the *G. coronatum* treatment, while *G. coronatum* could increase the weight dry total of 6.37g and significantly different from the treatment of *G. claroideum*.

Table 5. Effect of AMF (A) on spore count, colonization, total dry weight and number of leaves.

AMF Type (A)	Spores 4 mst	Colonization 4 mst (%)	Total Dry Weight (g)	Number of Leaves (strands)
A1	0a	18.33b	6.37a	40b
A2	1a	27.50a	5.70b	45a
Pr>F	0.0813	0.0253	0.0019	<.0001

Note: The mean value of the variable and the reference value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at 95% confidence. A1 (*G. coronatum*), A2 (*G. claroideum*).

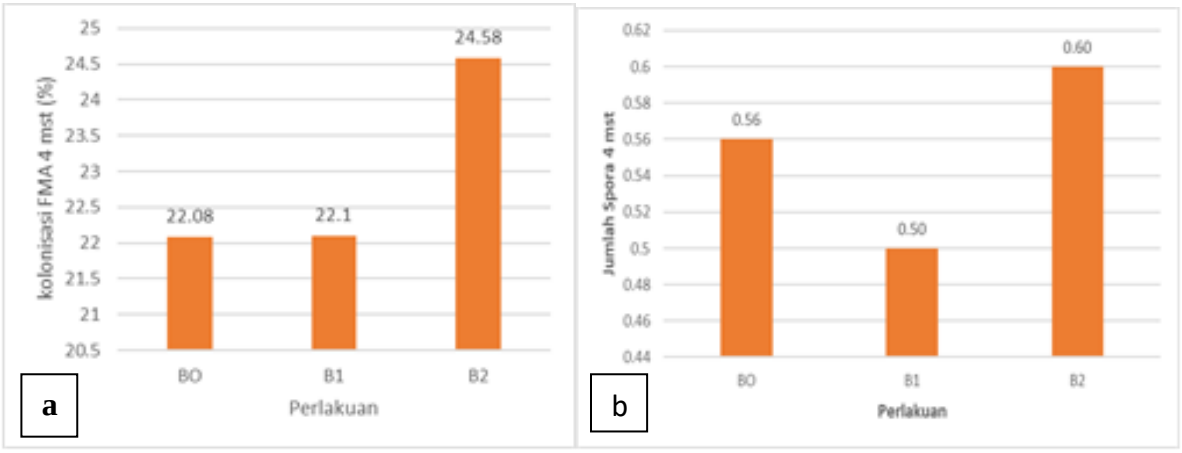
The Effect of Independent Nutrition AB Mix (B)

The results of the duncan test the effect of AB mix nutrient fertilizer treatment on shoot dry weight, total dry weight and number of leaves are presented in Table 6. Table 6 shows that the treatment with AB mix 5 ml nutrition resulted in the highest shoot dry weight (3.44) and was not significantly different in the treatment giving AB mix nutrition 2.5 ml except for treatment without AB mix nutrition. The variables of total dry weight and number of leaves produced the highest value in the 5 ml AB mix nutrition treatment of 6.97 g and 55 strands and were significantly different from the 2.5 ml AB mix nutrition treatment and without AB mix nutrition.

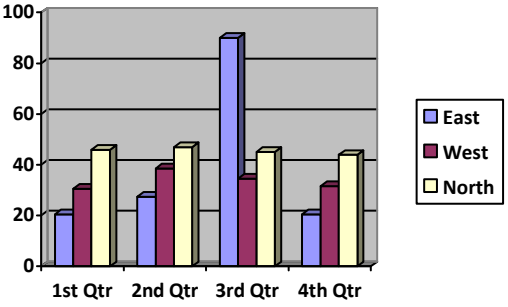
Table 6. Effect of Nutisi AB Mix (B) on shoot dry weight, total dry weight and number of leaves.

AMF Type (A)	Shoot Dry Weight (g)	Total Dry Weight (g)	Number of Leaves (strands)
B0	2.55b	4.97c	24c
B1	3.25a	6.17b	49b
B2	3.44a	6.97a	55a
Pr>F	0.0177	0.0019	<.0001

Note: The mean value of the variable and the reference value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at 95% confidence. B0 (control), B1 (AB nutrition mix 2.5 ml/ 1000 ml water), B2 (AB nutrition mix 5 ml/ 1000 ml water).



Fi pore count (1a) and AMF colonization (1b)



lysis of AB mix nutrition on the number of spores nization at 4 weeks after planting (Figure 1b), the 5 results of 1 spore and 24.58% colonization and was n . AB nutrition mix 2.5 ml and without giving AB m

Discu

tween AMF types and AB mix nutrient doses resulted in differences in AMF colonization and sporulation. The number of AMF spores and colonization continued to increase from month 1 to month 3 to the number of spores after drying. The treatment of *G. claroideum* had a tendency to increase sporulation more than *G. coronatum.* This is presumably because each type of AMF has different characteristics and abilities in responding to the addition of fertilizer and Glomus is classified as a species that is sensitive to the level of fertilization (Bhadalung et al., 2005; Husna et al., 2017).This is in line with previous research which said that Glomus spores were reported to grow well in all types of nutrients (Delvian, 2008). AB mix nutrition is a fertilizera complete inorganic compound that has low P and high N content so that it is easily absorbed by plants to help plant growth (Ginanjar et al., 2021).

The interaction of *G. claroideum* treatment with 2.5 ml of AB mix nutrition was the best treatment for AMF production after drying of 35 spores. However, the treatment of *G. claroideum* without AB mix nutrition was not significantly different from the treatment of *G. claroideum* with 2.5 ml of AB mix nutrition. This shows that AMF of the *G. claroideum* species can increase tolerance and the ability of plants to grow in acid soils due to the association of AMF colonization with plant roots and the ability of AMF to adapt to low pH conditions (Sieverding, 1991).

In addition to the number of spores, the effectiveness of AMF on host plants is also indicated by the ability of AMF to colonize host plant roots. Chalimah et al. (2007) explained that root colonization was the initial form of a symbiotic process between AMF and host plant roots. AMF colonization will provide a positive role in the provision of nutrients and water. Based on the results of the study showed that the type of *G. claroideum* with 2.5 ml of AB mix nutrition resulted in the highest colonization percentage of 69.16% compared to other types of treatment. Factit shows that type*G. claroideum*have a high degree of compatibility with host plants andshowed that each treatment had a different level of ability to colonize the roots of pueraria plants. This is in line with Smith and Read (2008), who explained that the difference in the percentage of colonization was due to differences in the type and level of compatibility between AMF and plant root systems.The ability of AMF to colonize roots will influence the growth of host plants. Kafid et al. (2015) explained that the higher the colonization of AMF on plant roots, it can be indicated that the more active mycorrhizal spores infect roots and expand the root absorption area for water and nutrients, so that plants can grow optimally.

In general, the results of research on the independent effect of 5 ml AB mix nutrition were able to increase shoot dry weight (g), total dry weight (g) and number of leaves (strands) with the highest yields being 3.44g, 6.97g and 55 strands, respectively. This shows that the 5 ml dose of AB mixed nutrition has a very good compatibility level with *P. javanica*, which is thought to be influenced by the ability of mycorrhizal roots to absorb nutrients and water. Rengganis (2013) explained that root development is due to the presence of AMF which enhances root interception in absorbing nutrients and water.

In general, plant dry weight continued to increase with the higher application of AB mix nutrition. This is because Glomus has larger intraradical hyphae and less extensive extraradical hyphae (Dodd et al., 2000). Larger intraradical hyphae allow a greater volume of nutrients to be transferred to the top of the plant for biomass formation whereas less extensive extraradical hyphae allow less carbon flow to the rhizosphere in colonized plants. As a soluble fertilizer,

In the variable number of leaves, the higher the nutrient AB mix, the more the number of leaves, this is caused by the function of AMF, namelyhaving the capacity to access nutrient sources both inorganic and organic in the soil, this role causes AMFdcan increase the absorption of various nutrients for plants, especially nutrient P for the formation of root nodules, leaves and plant roots (Smithdan Read, 2008; Souza, 2015). AMF symbiosis with plants is thought to be able to facilitate the provision of nutrients needed by plants so that they will grow better and produce a higher number of leaves than plants that are not inoculated with AMF (Hartoyo, 2012).

CONCLUSION

Based on the results of the research that has been done, it can be concluded that: Combination treatment of G. claroideum and administration of AB mix nutrition at a dose of 2.5 ml can give the best results in increasing the number of spores after drying of 35.46 spores and AMF type and AB mix nutrition were able to increase nodulation, shoot dry weight, root dry weight, nodule dry weight and number of leaves.

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