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The Effectiviness of Hormone Growth Promoting Fertilizers on Increasing The Production of Arbuscular Mycorrhizal Fungi Spores

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ABSTRACT. Arbuscular Mycorrhizal Fungi (AMF) is one of the fungi that forms a mutualism symbiosis with plants where there is a mutually beneficial relationship between the two parties and acts as a biological fertilizer that needs to be reproduced.AMF propagation is strongly influenced by the availability of nutrients, growing media and host plants. The purpose of this study was to determine the application of hormone growth booster fertilizer to increase the production of arbuscular mycorrhizal fungi spores.This research was conducted at the Plastic House of the Indonesian Mycorrhizal Association (AMI) Southeast Sulawesi Branch and the Laboratory of the Department of Forestry, Faculty of Forestry and Environmental Sciences, Halu Oleo University, Kendari during September – November 2022.This study was arranged based on a Completely Randomized Factorial Design. which consists of 2 factors. The first factor: AMF type which consists of two levels, namely Glomus coronatum and Glomus claroideum. The second factor: Fertilizer application consisting of three levels, namely without fertilizer application (B0), 2, and 5 ml/pot fertilizer (B2) with 3 repetitions. So there are 18 treatment combinations. Each treatment unit contained 9 plants so that the total number of plants was 162 plants. The results of this study indicate that the interaction treatment of G. coronatum and G. claroideum without fertilizer application can give the best results in increasing the number of spores for 3 months and in the treatment of G. coronatum with a fertilizer dose of 2, 5 ml gave the best results for increasing the number of spores after drying. Treatment of G. claroideum with a dose of 5 ml of fertilizer gave the best results in increasing the number of spores after drying.

Keywords: *Arbuscula Mycoroza Fungi, Glomus coronatum, Glomus claroideum, Growth-promoting Fertilizers*

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) is an organism derived from a class of fungi that can form a mutualism symbiosis with plant root systems where AMF obtains a carbon source from photosynthesis while plants obtain a supply of nutrients and water from AMF (Smith and Read, 2008). AMF comes from the endomycorrhizal group (Peterson, 2004). Endomycorrhizae are fungi that infect plant roots by entering the cortical cell tissue on plant roots. AMF has several structures. AMF structures commonly found in plant roots are external hyphae, internal hyphae, coil hyphae, vesicles and arbuscles (Smith and Read, 2008).

In general, AMF can symbiosis with 97% of higher plant roots (Smith and Read, 2008). AMF has a very important role in increasing plant growth (Mansur, 2010), especially on marginal land (Brundrett, 1991; Turk et al., 2006), this is due to its ability to absorb nutrients very well, both macro nutrients and micro. In addition, roots that have mycorrhizae can absorb nutrients in bound forms and those that are not available to plants in increasing plant productivity (Brundrett et al. 1996) and plant biomass (Husna et al., 2015). Based on the role that AMF has the potential as a biological fertilizer so that it can be developed as an

alternative to increase plant growth, including being able to carry out production activities (multiplication) of AMF spores.

AMF propagation can be done by growing AMF with host plants on zeolite media (Brundrett et al., 1996). AMF propagation is strongly influenced by the availability of nutrients, growing media and host plants (Smith and Read, 2008; Tuheteru, 2003). One effort to increase production is with proper maintenance and fertilization. The increase in AMF production can be done by fertilizing. The application of this fertilizer uses Hormonic Fertilizers, which are plant hormones. Hormonic fertilizers contain the following elements: organic growth regulators, especially: Auxin, Gibberellins and Cytokinins. The advantages of hormonal fertilizers accelerate the growth of leaves to become thick and wide and accelerate the development of stems (Sutedjo, 2010).

MATERIAL AND METHODS

Location and Time of Research. This research was conducted at the Southeast Sulawesi branch of the Indonesian Mycorrhizal Association (AMI) Plastic House. Its geographical position is at 3057'-3058' South Latitude, 122031'50" - 122032'0" East Longitude and the Forestry Laboratory Unit, Faculty of Forestry and Environmental Sciences, University of Halu Oleo Kendari, during September - November 2022.

Research Design. This study was arranged based on a Completely Randomized Factorial Design. which consists of 2 factors. The first factor: AMF type which consists of two levels, namely Glomus coronatum (A1) and Glomus claroideum (A2). The second factor: Fertilizer application consisting of three levels, namely without fertilizer application (B0), 2 ml/pot fertilizer (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 number of plants was 162 plants.

Research procedure

1. Growing media preparation
   2.07 mm zeolite rock, washed thoroughly to remove fine zeolite powder and existing impurities. Impure Zeolite rocks can have a negative impact on the development of AMF. Then sterilize with a sterilizer for 8 hours to remove possible pathogens. after that the zeolite rocks were soaked with a solution of hormonic growth accelerator fertilizer at a dose of 1 ml/liter of water for 24 hours (Nurrobifahmi et al., 2017)
2. Host Plant Preparation
   Sorghum used as a host plant was first soaked in a solution of bayclin at a dose of 2 ml/1 liter of water for 5 minutes, then rinsed with clean water for 3 times. The seeds are soaked in hot water at 50 degrees for ± 24 hours to break dormancy that may occur. After that, the seeds can be directly planted in the culture pot.
3. AMF propagation
   AMF propagation in this study used zeolite media. The media is filled in the container provided with a ratio of 150 grams of zeolite and 5 grams of inoculum containing AMF.
4. Maintenance

Culture maintenance activities include watering 2 times a day with a volume of 2 liters of water. Every 2 (two) weeks watering with harmonic fertilizer is carried out. Apart from watering, other maintenance activities include weeding and cutting dead sorghum leaves or branches.

5. Harvesting
   Harvesting activities are carried out once a month for three months.

Variables

1. Dry Weight

a) From each plant, shoots, roots 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 sports under a binocular microscope and written down the results.

3. AMF Colonization on Sample Plant Roots (%)

Observation of AMF colonization on plant roots was carried out through root staining techniques. Anatomical characteristics that characterize the presence or absence of AMF infection cannot be seen directly. unless the sampled roots are stained and viewed under a microscope. Therefore, staining of root samples in the staining technique method is very important in observing and identifying AMF infections in host plant roots. Choose fresh roots and wash with running water until clean. Root samples were soaked in 10% KOH solution until the roots became clear in color. If the root contains a lot of phenol, the solution will be dark brown in color. The KOH solution was then discarded and the root samples were washed in running water for 5-10 minutes. The root samples were then immersed in 2% HCL solution for 30 minutes. and in this process the roots will be white or pale. The 2% HCL solution was then removed by flowing it slowly.

Furthermore, the root samples were immersed in a staining solution (0.05% typan biue). Then the 0.05% trypan blue solution was discarded and replaced with 50% glycerol solution for the destaining process. Furthermore, observation activities to determine the

percentage of AMF colonization in root samples are ready to be carried out under a microscope.

Calculation of the percentage of root colonization using the slide method from Giovanneti and Mosse (1980) in Husna (2015). Randomly taken pieces of root that had been stained with a length of ± 1 cm as many as 10 pieces of root and arranged on a slide preparation. Root colonization is indicated by the presence of hyphae, vesicles, arbuscles or one of the three. The method to be used for cleaning and staining the sample roots is the method from Kormanik and McGraw (1982) in Brundet et al., (1996).

$$\%\ Kolonisasi\ = \frac{\Sigma \text{ bidang pandang terkolonisasi (+)}}{\Sigma \text{ total bidang pandang}} \text{x100\%}$$

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 variance (test F) the treatment of the types of Arbuscular Mycorrhizal Fungi (FMA) and the application of Hormonic Growth Promoter Fertilizers are presented in table 1. Table 1 shows that the interaction between AMF types and Hormonic Fertilizers (A*B) has a very significant effect on 1 month spores, 3 months and spores after drying, shoot dry weight, roots and total. As well as not having a significant effect on 2 month spores, 1,2,3 month colonization and number of leaves.

The influence of AMF type factors (A) had a very significant effect on the number of three month spores, spores after drying and had a significant effect on the first month spores, second month colonization and number of leaves. On the variable number of spores in two months, colonization in one month, three months, dry weight of shoots, roots, total had no significant effect. Hormonic fertilizer factor (B) had a very significant effect on the number of spore months one, two, three, spores after drying, and number of leaves. Meanwhile, colonization in months one, two, three dry weight of shoots and roots dry weight and total dry weight had no significant effect.

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Table 1.Recapitulation of the effect of various types of FMA andapplication of Hormonic Growth Promoter Fertilizer to research variables

Observed variables	Treatment			KK
	FMA type	Fertilizer Hormonics	A*B	
	A	B		
Number of Spores Month 1	*	**	**	22.98
Number of Month Spores 2	mr	**	mr	7.85
Number of Month Spores 3	**	**	**	4.81
Spores after drying	**	**	**	4.42
Moon root colonization 1	mr	mr	mr	13.91
Moon root colonization 2	*	mr	mr	8.33
Moon root colonization 3	mr	mr	mr	7.90
Number of Leaves	*	**	mr	9.01
Shoot dry weight	mr	mr	**	3.20
Root dry weight	mr	mr	**	3.48
Total dry weight	mr	mr	**	3.32

Description: ** : very significant effect (P≤0.01)
* : significant effect (P≤0.05)
tn : no significant effect (P>0.05)

The Interaction Effect of AMF Type Treatment and Hormonic Growth Promoter Fertilizer

A. Number of Spores

The results of the Duncan test for the effect of the interaction between AMF and Hormonic fertilizers on the number of spores are presented in Table 2. Table 2 shows that the AMF treatment with *G. claroideum* type with 2.5 ml of fertilizer produced the most spores in month 1 of 18 spores and not significantly different from the other treatments but significantly different from the Glomus claroideum treatment with 5 ml of fertilizer. Treatment of the AMF type *G. coronatum* without fertilizer application At month 3 produced the most 45 spores and was not significantly different from the treatment of *G. claroideum* without fertilizer application. The treatment of *G. Coronatum* with 2.5 ml of fertilizer produced the most spores after drying with 42 spores and was not significantly different from the other treatments.

Table 2. The effect of the combination of AMF and Hormonic fertilizers on the number of spores/5 gram of inoculum

Treatment Number of spores (gr)				
FMA	**Hormonics (ml)**	**1st month**	**3rd month**	**After drying**
Glomus	Control	14a	45a	38b
Coronatum	2.5	16a	41b	42a
	5	14a	22d	24c
Glomus	Control	15a	45a	38b
Claroideum	2.5	18a	37c	37b
	5	3b	37c	38b
Pr > F		0.0010	<.0001	<.0001

Note: The mean value of the variable and the comparison value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at the 95% level of confidence.

Dry Weight of Shoots, Roots and Total Plants

The results of the Duncan test on the interaction of AMF and Hormonic fertilizers on dry weight of shoots, roots and total plant are presented in Table 3. Table 3 shows that AMF types*Glomus coronatum*with the application of 5 ml of hormonal fertilizer produced the highest shoot dry weight with a value of 6.81g and was significantly different from other treatments. On the type of FMA*Glomus coronatum*with the application of 5 ml of horminic fertilizer produced the highest root dry weight with a value of 6.86g and was significantly different from other treatments. total dry weight of AMF species*Glomus coronatum*by giving 5 ml of horminic fertilizer also produces the highest total dry weight i.e13.68g and significantly different from other treatments.

Table 3. Combination treatment of AMF and hormonal fertilizers for dry weight

Treatment Dry weight(g)				
FMA	**Hormonics (ml)**	**Shoots**	**Root**	**Total**
Glomus	Control	6.07c	6.08c	12.16c
Coronatum	2.5	6.49ab	6.49abc	12.98ab
	5	6.81a	6.86a	13.68a
Glomus	Control	6.37ab	6.71ab	13.42ab
Claroideum	2.5	6.64ab	6.64ab	13.28ab
	5	6.37bc	6.37bc	12.75bc
Pr > F		0.0112	0.0160	0.0131

Note: The mean value of the variable and the comparison value of Duncan's multiple range test (DMRT), the same letter in the same column does not differ at the 95% level of confidence.

The influence of the independent factor of FMA

Duncan's test results for the effect of independent FMA treatment are presented in Table 4. Table 4 shows that AMF did not significantly increase 2 month spores, 1,2,3 month

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colonization, and number of leaves but there was a tendency to increase 2 month spore, 1,2,3 month colonization , and Number of Leaves.

Table 4. Influence of FMA independent factors

Treatment	Number of Spores (gr)	Root Colonization (%)			
FMA	2nd month	1st month	2nd month	3rd month	Number of Leaves
G, coronatum	26	58.61	73.05	79.44	44.88
G, claroideum	27	57.72	69.94	76.94	40.22

Fertilizer independent factor influence

The results of the Duncan test on the effect of Fertilizer Independent treatment are presented in Table 5. Table 5 shows that the Fertilizer treatment did not significantly increase 2 month Spores, 1, 2, 3 month Colonization, and Number of Leaves but there was a tendency to increase 2 month Spores, 1, 2 month Colonization, 3, and Number of Leaves.

Table 5. Effect of Independent Fertilizer Factors

Treatment	Number of Spores (gr)	Root Colonization (%)			
Fertilizer (ml)	2nd month	1st month	2nd month	3rd month	Number of Leaves
Control	31	61.25	71.66	79.16	24.33
2.5	31	57.91	71.66	78.75	48.66
5	16	54.83	66.66	76.66	54.66

Discussion

The interaction treatment of AMF and Hormonic fertilizers had a very significant effect on the number of spores (1-3 months and after drying). The number of AMF spores for Glomus claroideum and Glomus coronatum types without fertilizer application at 3 months had the highest number of spores compared to other treatments of 45 spores. This is because the higher the dose of fertilizer, the sporulation of AMF decreases. This is because each type of AMF has different characteristics and abilities in responding to the addition of fertilizer given and the AMF of the Glomus type is classified as a species that is sensitive to the level of fertilization (Bhadalung et al., 2005).

The number of spores in the first month and after drying showed different results, where the 1 month spores in the *G cloroideum* treatment and the administration of 2.5 ml of hormonal fertilizer produced the highest number of spores of 18 spores. and the spores after drying in the *G. coronatum* treatment and the administration of hormonal fertilizers at a dose of 2.5 ml resulted in the highest number of spores of 42 spores. In the interaction between the AMF type treatment and the administration of 2.5 ml of spore monthly hormone fertilizer, the highest number of spores was found in both the AMF types of *G. coronatum* and *G*

cloroideum. This is because the lower the ability of the host plant and the growing media to bind water and nutrients, the greater the increase in the number of spores. Conversely, the higher the ability of the host plant and growing media to bind water, the lower the number of increased spores (Trisnayanti et al., 2021). In line with this, Samsi et al., (2017) also suggested that the higher the fertilizer value, the lower the number of spores.

Besides the fertilizer factor, there are other factors that affect spore production. Among them are temperature and light factors. Excess shade, especially for light-loving plants, can reduce root infection and spore production, besides that the response of plants to mycorrhizal fungi will be reduced. This is due to the inhibition of growth and internal development of hyphae in roots which results in limited development of external hyphae in the rhizosphere (Setiadi, 2001 in Simamora, 2015). Temperature affects infection, namely on the development of spores, penetration of hyphae in root cells and development in the root cortex, besides that temperature also affects resistance and symbiosis. The higher the temperature the greater the formation of colonization and increased spore production. Simamora (2015) stated that AMF will reach maximum growth at 30oC, but mycelial colonization on the root surface is best at 28-35oC. While the best sporulation and vesicle growth at 35oC.

The results of the study are in Table 3showed that the interaction of AMF Glomus coronatum and hormonal fertilizers increased shoot dry weight and root dry weight by 6.81g and 6.86g. The results of this study are in line with the research of Irianto (2015) which stated that Glomus coronatum inoculation increased shoot dry weight and growth of three-year-old suren seedlings.

month in the nursery. As a soluble fertilizer, fertilizer is one of the elements needed by plants and can be absorbed by AMF which can increase the growth of roots and shoots which has implications for increasing the total dry weight of a plant because it can increase the absorption of nutrients and water thereby increasing the rate of photosynthesis (Smith and Read, 2008).

The increase in dry weight (shoots and roots) of the Glomus FMA species was effective because it had larger intraradical hyphae and less extensive extraradical hyphae (Dodd et al., 2000). Larger intraradical hyphae allow a greater volume of nutrients to flow to the top of the plant for biomass formation, while less extensive extraradical hyphae result in less carbon flow to the rhizosphere in colonized plants (Husna et al., 2017).

The results of the research in tables 4 and 5 show that each treatment has a different level of ability to colonize plant roots. This is in line with opinionSmith 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. Setiadi (1989) explains that kRoot colonization by AMF is determined by the level of effectiveness and suitability between AMF and host plants.

Powell and Bagyaraj (1984) in (Husna 2015) explained that the colonization and production of AMF spores is related to plants, AMF types and environmental conditions such as sunlight, temperature and humidity. This is in line with what was stated by Setiadi (1989) that in general AMF species in carrying out the colonization process require a temperature of 26 – 32^{o}C. The compatibility of a particular AMF species with the host under the right environmental conditions is very important for AMF production.

The ability of AMF to colonize roots will influence plant growth. 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 this study, observation of AMF colonization on host plant roots was also carried out by observing the structure of AMF that had formed on host plant roots in the form of vesicles, arbuscules, internal hyphae, and external hyphae. This is in line with the statement of Setiawan (2011) explaining that if there is an infection in the roots of a plant, there will be parts of the AMF which include hyphae, arbuscles and vesicles.

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