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Arbuscular Mycorrhizal Fertilizer Powder Formulation (*Glomus mosseae*) As Bio Fertilizer Raw Materials

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Abstract:- The current formulations of mycorrhizal biofertilizers are of low quality, have not utilized local waste raw materials, have low ability to inoculate, have low contact area with plant roots, and have low inter-ion bonds, making it difficult to attach to roots and easily separated from roots, thereby slowing growth, infection process and short shelf life. This study aims to make arbuscular mycorrhizal fertilizer formulations into powder using coconut charcoal as a raw material for the manufacture of biofertilizers that are easily available, long-lasting, and of good quality. The experiments were conducted in greenhouses and agricultural microbiology laboratories, Faculty of Agriculture, University of Mataram, Mataram, West Nusa Tenggara, Indonesia. Parameters measured were moisture content, number of spores per 20 g of powder, colonization percentage and shelf life. The results showed that the substrate formulation of *G. moseae* isolate MAA-01 as much as 75% was mixed with 25% coconut charcoal powder until homogeneous, passed a 10 mesh sieve and packed in aluminum foil had a spore count of 1500 per 20 g powder, a colonization percentage of 90%, water content 4.973% db, and shelf life of 6 months. The resulting arbuscular mycorrhizal biofertilizer powder formulation has high quality inoculum potential, high number of spores and colonization. This product is suitable for farmers because the technology is simple, easy to manufacture, and can be used to increase crop production on marginal land.

***Keywords**: Arbuscular Mycorrhizae; Biofertilizer Raw Materials; Powder Formulations.*

I. INTRODUCTION

Arbuscular mycorrhizal biofertilizers are not durable when stored at room temperature, they are obligatory symbionts that require the presence of host plants, and their production does not utilize local waste materials, which are widely available and environmentally friendly. Arbuscular mycorrhizal biofertilizers that have been made only have a shelf life of 30 days at room temperature. To increase the shelf life and economic value of this biological fertilizer, it is necessary to carry out adequate processing. One way is to make arbuscular mycorrhizal powder formulations that can be used as raw materials for biological fertilizers.

Many preparations have been made of arbuscular mycorrhizal fungi powder formulations, including the manufacture of arbuscular mycorrhizal biofertilizer powders using the method Trip and Sieving [1], mycorrhizal granulation process method with granule machine [2], and the method of formulation of tablets with excipients gypsum and clay [3]. However, this biological fertilizer still has several weaknesses, including not utilizing local raw material waste, low ability to inoculate, low contact area with plant roots, and low inter-ion bonds, making it difficult to attach to roots and easily separate from roots, slowing down the infection process and short shelf life.

This fact shows that there is still a need to develop raw material formulation methods that can improve the characteristics of physical and chemical biological fertilizers and extend the shelf life of the product. The methods that can be used include implementing technology to improve the formulation process for the provision of raw materials in powder form. This technology is directed at improving the inoculum potential of biofertilizers, considering that the characteristic to be improved is the potential of the inoculum in the form of spore count and percentage colonization, water content, and storage capacity.

Formulation in powder form have a high inoculating speed because they have a wider contact area with plant roots than granules. Inter-ionic bonds are also high, so it is easily attached to the roots and not easily separated; it can accelerate the infection of the roots.

The application of arbuscular mycorrhizal biofertilizer powder can improve the physical characteristics of arbuscular mycorrhizal biofertilizers, such as increasing the number of spores per gram of soil, the percentage of root colonization and good inoculum potency when inoculating plants, to increase plant growth and yield [4], [5].

The search carried out through the website http://ep.espacenet.com and https://pdki-indonesia.dgip.go.id/index.php/paten showed that arbuscular mycorrhizal biofertilizer powder formulations are made of several components, such as organic matter and clay. However, this formulation still has weaknesses; for example, it is difficult to crumble, and it is still difficult to unite with the roots of plants that will be infected after being applied. Other research results revealed that arbuscular mycorrhizal biofertilizer powder was produced through several processing stages: collection, screening, isolation, formula preparation, and storage. Furthermore, other research results found that the formulation of a mixture of arbuscular mycorrhizae with *Massilia sp.* RK4 can increase plant growth. However, this mixed formulation still needs to improve: it is limited to use only in soil conditions with high salt salinity.

Some of the previous formulation findings still have some weaknesses, such as low ability to inoculate, low contact area with plant roots, and low ionic bonds, making it difficult to attach to roots and easily separated from roots thereby slowing down the infection process. The advantage of our research finding of Arbuscular Mycorrhizal Fertilizer Powder Formulation is that this biological fertilizer powder formulation product has the potential for high-quality inoculums, increases the ability of inoculating roots and can increase plant growth and yield. In addition, this product formulation has a longer shelf life, which is around 6 months [6].

The production process of conventional arbuscular mycorrhizal biofertilizers requires a long time, making it difficult to obtain during the growing season and when needed. Hence, the manufacture of arbuscular mycorrhizal fertilizer into powder as a raw material for the manufacture of biofertilizers, which can be easily available because it is made from local coconut charcoal derived from coconut shell waste, which is abundant and available throughout the year, is important.

II. MATERIALS AND METHODS

A. *Experimental Site*

The experiments were conducted in greenhouses and agricultural microbiology laboratories, Faculty of Agriculture, University of Mataram, Mataram, West Nusa Tenggara, Indonesia. There is a culture substrate (*G. moseae* isolate MAA-01) and coconut charcoal powder as ingredients for the formulation of arbuscular mycorrhizal biofertilizer powder.

B. *Experimental Design*

Before the research was carried out, an exploration of indigenous arbuscular mycorrhiza was first carried out in the Village of Akar-Akar, North Lombok. The isolate was coded MAA-01 with the species *Glomus mosseae*. Further experiments were carried out in greenhouses and laboratories [7]. Research begins by multiplying the MAA-01 arbuscular mycorrhizal isolate in culture pots with maize as the host plant.

The second stage was the formulation of arbuscular mycorrhizal fertilizer by mixing 1500 g of mycorrhizal inoculant with 500 g of coconut charcoal powder as a carrier (3:1), then stirring until a dough was formed, then tested the number of spores with the wet sieving and decanting method [8], [9], and mycorrhizal colonization was carried out by the method of cleaning and staining [10], moisture content by heating method [11], and shelf life by storing arbuscular mycorrhizal biofertilizer powder in aluminum foil packaging at room temperature with observed changes in the number of spores every week. The research process flowchart is described in Figure 1.

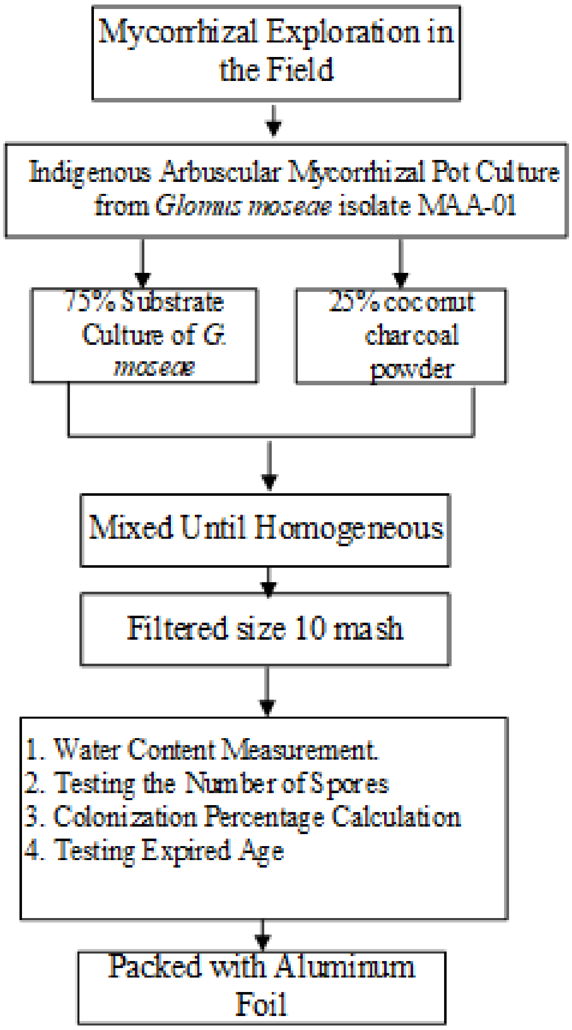

Fig. 1 Research process flowchart

C. *Data Interpretation*

Data was collected by direct observation, calculation, and description, then compared with various previous research literature, both from national and international journals as well as online databases such as Google Scholar, Researchgate, and the Directory of Open Access Journals. The data obtained was then analyzed using a descriptive method, namely by explaining the results of the discussion and then drawing conclusions.

III. RESULTS AND DISCUSSION

The manufacture of arbuscular mycorrhizal fertilizers produces powdered fertilizers with physical and chemical characteristics that can be used as raw materials for the manufacture of mycorrhizal biofertilizers and are easily available without having to wait long.

The process of making arbuscular mycorrhizal fertilizer formulations was initiated by multiplying MAA-01 arbuscular mycorrhizal isolates in culture pots with a ratio of

5 kg of mixed soil media and 5 kg of sterile manure (1:1). These culture pots were then inoculated with 40 g of the arbuscular mycorrhizal isolate MAA-01 per culture pot. Then this culture pot is planted with corn as the host plant [12]. These plants are then maintained for three months. Furthermore, after three months, the culture pots are harvested and dried until the moisture content reaches 10%. The roots of the harvested corn are finely blended, then mixed evenly with the sifted soil with a 2 mm diameter sieve. The arbuscular mycorrhizal inoculant obtained was a mixture of mycorrhizal spores, mycorrhizal hyphae, infected roots, and soil containing mycorrhizae.

Formulation of arbuscular mycorrhizal fertilizer by mixing 1500 g of mycorrhizal inoculant with 500 g of coconut charcoal powder as a carrier (3:1), then stirring until a dough is formed. The mixture was then sieved on a ten mesh sieve by shaking the sieve until a wet powder was formed. Furthermore, this wet powder is dried for 12 hours. After the mycorrhizal powder, the arbuscular powder was dried and tested for the number of spores, mycorrhizal colonization, moisture content, and shelf life. Arbuscular mycorrhizal fertilizer powder is packed on aluminum foil. With the embodiment of this research process, a powder formulation of arbuscular mycorrhizal fertilizer was produced that had a spore count of 1500 per 20 g of powder, a colonization percentage of 90%, a moisture content of 4.973% db, and a shelf life of 6 months, as shown in Table 1. Mycorrhizal spores and vesicles as shown in Figure 2.

Table 1. Test results of mycorrhizal fertilizer powder

No	Test Type	Test results
1	Number of spores	1500 spores/20 g
2	% colonization	90%
3	Water level	4,973 db
4	Shelf life	6 months

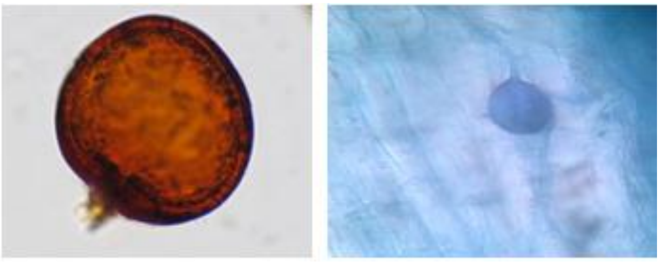

Fig. 2. Spores and vesicles mycorrhizal *G. mosseae*

An agricultural commodity farming system is very complex, dynamic, and constantly interacting with other systems. A systemic approach is required for the success of dryland commodity farming research in an effort to find strategic keys for development and, at the same time, preserve it. Directed agrotechnology engineering for various commodities is strongly supported by research results at various agricultural research and development centers and other related agencies, as well as the practical experience of community leaders.

The gap between the community's mastery of commodity agrotechnology and directive agrotechnology will determine the scale of priority for commodity development. Failure to integrate information on community agrotechnology mastery with information on land resources could result in the failure of the dryland commodity development program. It has been recognized that chemical plant fertilizers produced by industry (factories) have contributed significantly to increasing agricultural production, so farmers are very dependent on these fertilizers.

However, this type of fertilizer has recently become a very serious national problem because it is often unavailable in the field, so it has become a social problem with demonstrations, hijacking of fertilizer transportation, and so on. To overcome farmers' dependence on chemical fertilizers, it is necessary to make a breakthrough by using biological fertilizers, which are cheaper and, of course, available in nature. One way that can be done is by using mycorrhizae, which so far have not been studied in depth in Indonesia.

The results of this research have created a formulation process for indigenous arbuscular mycorrhizal biofertilizer from *Glomus mosseae* isolate MAA-01 from Akar Akar Village, North Lombok, with charcoal powder carrier from coconut waste.

The process of making arbuscular mycorrhizal powder formulation begins with the multiplication of indigenous arbuscular mycorrhizal isolates from *Glomus mosseae* isolate MAA-01 with charcoal powder carriers from coconut waste in culture pots with 5 kg of soil mixed media and 5 kg of sterile manure (1:1). These culture pots were then inoculated with 40 g of arbuscular mycorrhizal isolate MAA-01 per culture pot. Then this culture pot is planted with corn as the host plant. These plants are then maintained for three months. Furthermore, after three months, the pot culture is harvested, then air-dried until the moisture content reaches 10%. The roots of the harvested corn are finely blended, then mixed evenly with the sifted soil with a 2 mm diameter sieve. The arbuscular mycorrhizal inoculant was a mixture of mycorrhizal spores, mycorrhizal hyphae, infected roots, and soil containing mycorrhizae.

The dough was prepared by weighing 1500 g of mycorrhizal inoculant and then adding 500 g of coconut charcoal powder as a carrier (3:1). This mixture is stirred with a mixer until a homogeneous dough is formed. Furthermore, this mixture is sieved on a 10-mesh sieve by shaking the sieve until it forms a powder. This powder is stored in a tin and then air-dried for 12 hours to produce a powder moisture content that is safe for storage.

The measurement of moisture content in the dry powder of mycorrhizal biofertilizer was done by heating method [11]. A sample of 5 g was put into a cup whose weight was known, then dried in a vacuum oven at a temperature of $70^{O}C$ until a constant weight is obtained. After calculating, the water content of the powder is 4.97% db.

Testing the number of spores was carried out by wet sieving and decanting, according to Brundrette et al. [8]. A sample of 100 g is immersed in a glass container for 15 minutes with 400 ml of water. After that, it is shaken with an electromagnetic stirrer for 6 minutes, then filtered in stages with a lattice diameter of 300 µm, 106 µm, 53 µm, and 38 µm. The results of the last filter were washed with water using a hand sprayer until clean. The filter results in the 38 µm fraction were put into a tube centrifuge, and 45 ml of water was added, then rotated in a centrifuge at 2000 rpm for 15 minutes. Supernatant was taken, added 25 ml of 50% sugar solution was, and then rotated again in an in centrifuge at 2000 rpm for 10 minutes, the Daniels and Skipper procedure [9]. Supernatant was sieved using filtered 38 µm then poured into an Erlenmeyer flask to be filtered using filter paper. Spores produced on filter paper were placed in a Petri dish to count the population per 100 g of soil under a stereo microscope with 40x magnification. The calculation results obtained an average number of spores per 100 g of soil, 7500 spores.

Calculation of the percentage of colonization was carried out by cleaning and Staining from Kormanic and Graw [10], which is modified. The roots were washed and cut into about ± 1 cm, then soaked in a 10% KOH solution-water bath for 30 minutes. The roots were rinsed again in running water, and the remaining KOH solution was discarded. After that, the roots were rewashed thoroughly and soaked in 5% HCl for 2 minutes. For coloring, the roots are soaked in trypan blue lactoglycerol 0.05% and heated to boiling for ± 3 minutes. Lactoglyserol trypan blue was discarded, and the roots were stored in a film bottle containing lactoglyserol 0.05%. The colored roots are observed below the microscope to calculate the infection rate. The results of the calculation obtained an average colonization percentage of 90%.

The shelf life of arbuscular mycorrhizal biofertilizer powder was calculated by storing it in aluminum foil packages at room temperature. Observations of changes in the number of spores were carried out every week. The results showed that arbuscular mycorrhizal biofertilizer powder had decreased the number of spores on the 27th week of storage. This is because arbuscular mycorrhizae are obligate symbionts and can only survive if a host plant exists. It is suspected that there was a decrease in the number of spores at week 27 because mycorrhizae did not receive a carbon supply due to the absence of host plants [13].

This biological fertilizer powder contains organic ingredients from cow manure, which have an important role in improving soil's physical, chemical, and biological properties. The role of the physical soil, among others, is as an adhesive material between soil particles to unite into soil aggregates, increasing soil porosity and increasing soil holding capacity [14]. Adding organic ingredients is expected to change the soil's structure from single-grained to lumpy, thus increasing the degree of structure and aggregate size or increasing the structural class from fine to medium or coarse [15]. In addition, the provision of organic matter can make a real contribution to cation exchange capacity (CEC) land. Around 20–70% of soil CEC is generally sourced from colloids, so there is a correlation between organic matter and soil CEC [16]. CEC organic matter is obtained from negative cow manure. The main source of negative charge-cow manure is mainly derived from carboxyl and phenolic groups [17].

The addition of organic matter in the form of cow manure to the production of biological fertilizer powder also utilizes waste charcoal coconut. Charcoal coconut contains activated carbon whose carbon atom configuration is freed from bonding with other elements, and its cavities or pores are cleaned of other compounds or impurities so that the surface area and active center become wider or increase the adsorption power of liquids and gases. The ability of charcoal coconut is expected to retain water through its large adsorption power. In addition, charcoal is hygroscopic and can absorb more water [18].

The beneficial interactions between roots and soil microbes also play a significant role in manufacturing this biological fertilizer powder. Beneficial soil microbes such as Arbuscular Mycorrhiza (AM) can increase the supply of plant nutrients and symbiotically interact with plant roots to absorb nutrients [19,20]. Mycorrhizal inoculation can also increase the efficiency of plant roots in absorbing nutrients by 2.3 times [21]. Inoculation with seed coating with indigenous mycorrhizae can increase growth, crop production, plant N and P uptake, and nutrient availability in the corn-sorghum cropping pattern on dry land [22].

The results of this study have advantages, including the process of making powder formulations of mycorrhizal biological fertilizers using only simple technology in the form of a sieve and dryer. The tool used for drying the powder is also simple, namely in the form of a baking sheet placed in the open air to dry it so it can be applied to farmer-scale industries. While the raw material for manufacturing mycorrhizal biological fertilizer powder is widely available, it comes from coconut shell waste, which is abundantly available throughout the year, is environmentally friendly, and can increase plant growth and yields under extreme conditions on dry land.

IV. CONCLUSION

The results showed that the substrate formulation of *G. moseae* isolate MAA-01 as much as 75% was mixed with 25% coconut charcoal powder until homogeneous, passed a 10 mesh sieve and packed in aluminum foil had a spore count of 1500 per 20 g powder, a colonization percentage of 90%, water content 4.973% db, and shelf life of 6 months. The resulting arbuscular mycorrhizal biofertilizer powder formulation has high quality inoculum potential, high number of spores and colonization. This product is suitable for farmers because the technology is simple, easy to manufacture, and can be used to increase crop production on marginal land.

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