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Manufacturing Process for Ameliorant Plus Mycorrhizal Powder Using Local Raw Materials for Soil Improvement

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Abstract:- The development of ameliorant powder enriched with indigenous arbuscular mycorrhizae for soil amendment has been limited. This study presents a formulation process for creating a high-quality ameliorant powder using local raw materials. The powder incorporates a culture of *Glomus mosseae* isolate MAA-01, which constitutes 25% of the mixture, combined equally with compost powder, cattle manure powder, and rice husk charcoal powder. This formulation results in a product containing 2500 spores per 20 g of powder, achieving a 95% colonization rate, a moisture content of 5.002 % db, and a shelf life of 12 months. Laboratory tests confirm that this ameliorant powder significantly enhances soil fertility, as well as plant growth and yield, particularly in suboptimal land conditions. The production method is straightforward, making it highly suitable for local farmers seeking to improve agricultural productivity.

***Keywords**:- Arbuscular Mycorrhizae; Soil Amendment; Sustainable Farming; Plant Yield Enhancement.*

I. INTRODUCTION

One of the key components of integrated plant management on suboptimal lands involves improving the soil's nutrient content by applying amendments to enhance plant production [1]. An ameliorant, or soil conditioner, is a substance added to soil specifically to improve the root environment for plant growth. The purpose of providing these amendments is to supply nutrients, reduce soil acidity, facilitate the binding or absorption of cations carried by water flow, and increase soil fertility on suboptimal lands [2].

The ameliorant materials commonly used for cultivating plants on suboptimal lands include locally sourced raw materials that are readily available in the field and typically found as unused waste. These materials include cattle manure, rice husk charcoal, and compost [3].

Cow manure is used as an ameliorant because it is readily available, cost-effective, can raise soil pH, and contains essential elements such as nitrogen (N), phosphorus (P), and potassium (K), which may substitute for some nutrients. Before application, cattle manure is first fermented with a starter to act as a bioactivator. Although cow manure naturally decomposes into a usable form of manure, this process can be quite slow. To expedite the composting process, a starter can be used. The composition of dry cattle manure typically includes 0.40% nitrogen, 0.20% P_2O_5 (phosphate), and 0.10% K_2O (potash) [4].

Adding rice husk charcoal to soil has the potential to enhance plant growth, nutrient retention, and nutrient availability. This improvement is linked to an increase in cation exchange capacity (CEC) and the surface area of the charcoal [5]. The porous nature of charcoal enhances the circulation of water and air within the soil, thereby expanding the reach of plant root systems [6]. Charcoal's many pores can store nutrients, which are then released gradually according to the plants' nutritional needs [7], ensuring that nutrients are not easily leached from the soil [8]. Charcoal is more effective at binding aluminum (Al) than at directly providing nutrients [9]. Moreover, adding charcoal has been shown to raise soil pH [10], enhance nutrient retention [11], reduce levels of exchangeable Al [12], and increase the availability of phosphorus (P) [13]. Furthermore, incorporating charcoal into the soil can minimize the leaching of basic cations, nitrogen (N), phosphorus (P), potassium (K), and microelements [14].

Compost acts as a soil improver similar to humus. Mature compost releases macro and micronutrients more slowly than inorganic fertilizers because it is both volatile and slow-release, ensuring long-lasting effects [15]. Additionally, compost enhances soil structure, increases the soil's water retention capacity, and serves as an adhesive that helps soil particles bind together into aggregates [16].

The formulation of arbuscular mycorrhizal fungi for breeding has been extensively developed, including methods such as dolomite granulation [17], the use of bio-activators [18], fermentation with starters like bio-activators [19], and seed coating processes that enhance mycorrhizae using starch glue adhesive [20]. However, these formulations still exhibit several drawbacks, including low water-holding capacity, high application volume, high water content, short shelf life, and low nutrient availability, all of which do not yield the optimal response in plant performance.

This fact underscores the ongoing need to employ raw material formulation methods that enhance the physical and chemical properties of soil improvers and extend the product's shelf life. Applicable methods include

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technological advancements in the formulation process that allow for the provision of raw materials in powder form. This technology is designed to improve the quality of the improver plus mycorrhizae, focusing on enhancing characteristics such as the inoculum potential, indicated by the number of spores, the percentage of colonization, water content, water retention capacity, and storage capacity.

The powdered form of the improver boasts a high water-holding capacity, rich nutrient content, and rapid inoculation speed due to its larger contact area with plant roots compared to granules. It also exhibits strong ionic binding, which facilitates easy adherence to roots, enhances water retention, and prevents easy detachment. This contributes to accelerated root colonization and efficient delivery of nutrients and water to the plants.

The test results demonstrate that applying soil amendment powder enriched with arbuscular mycorrhizae enhances the physical, chemical, and biological properties of the soil. Additionally, it provides macro and micronutrients in a slow-release form, increases the number of spores, boosts the percentage of root colonization, and offers high inoculum potential.

Searches were conducted through the websites http://ep.espacenet.com and https://pdki-indonesia.dgip.go.id/index.php/paten. Information was obtained that an improver formulation containing the "purun tikus" herb (sweet olives) has been registered under Indonesian patent number IDP000048840. This patent discloses an ameliorant formula designed to control iron poisoning in rice plants on acid sulfate land, consisting of 40-50% "purun tikus" grass, 40-50% rice straw, 2.5-7.5% chicken manure, and 2.5-7.5% dolomite.

Another patent search, patent number IDS000004255, reveals a sub-biochemical formula and its manufacturing process as a marginal soil improvement material for soils like Ultisol. The formula comprises subbituminous powder and biochar from waste young coconut, each at a rate of 10 tons per hectare (50% subbituminous + 50% biochar), specifically tailored for Ultisol marginal soils.

Further, patent number IDS000003447 details a fly ash suspension formulation (FASF) that involves mixing fly ash raw materials with a low concentration of weak acid at room temperature. This formulation alters the structure of fly ash, which is hazardous due to the presence of nano-sized particles, into a safer dosage form. The addition of low-concentration ionic or anionic surfactants enhances the solubility of minerals in the emulsion. The application of the FASF solution improves soil properties by increasing soil pH, total dissolved substances (TDS), and electrical conductivity (EC). The use of FASF has shown to enhance the growth of sorghum plants, as evidenced by increases in plant height, the number of leaves per plant, leaf length, and root growth improvement.

Additional patent search results include information from a United States patent application with publication number UA79889(U). This patent describes a method for neutralizing toxic aluminum in soil by applying lime. Another U.S. patent, publication number UA75109 (U), details methods for increasing the capacity of agricultural products. One such method involves using absorbent materials, specifically zeolites, which are uniformly spread in the soil during autumn plowing to enhance the yield capacity of agricultural land.

Several previous innovations have displayed weaknesses, such as low water holding capacity, insufficient soil nutrient content, slow inoculation speed, limited contact area with plant roots, and weak ionic bonding. These issues make it difficult for the materials to adhere to the roots and can lead to easy detachment, consequently slowing the infection process. The advantage of the proposed innovation is that this ameliorant in powdered biological fertilizer form has the potential to produce high-quality inoculum, enhance root inoculation capacity, and boost plant growth and yield. Additionally, this formulated product has a longer shelf life—approximately 12 months, compared to the standard 3 months.

The production process of conventional soil improvers is quite time-consuming, making it challenging to obtain them during the planting season and to have them readily available when needed. This invention introduces a powdered improver formulated from local raw materials derived from plant waste residues, which are available throughout the year. This powder formulation boasts a shelf life of 12 months. Moreover, it is more effective at enhancing the physical and chemical characteristics of the soil compared to conventionally produced soil conditioners. Another benefit of this product is that it utilizes abundant, locally sourced plant waste, available year-round.

II. MATERIALS AND METHODS

A. Purpose of the Invention

A brief description of the purpose of this process is to manufacture a powdered soil improver. The goal is to produce an improver in powder form that exhibits desirable physical and chemical characteristics, serving as a raw material for creating soil improvers. This format ensures that the product is readily available without long wait times.

B. Formulation Development Process

The process of preparing the ameliorant formulation with added arbuscular mycorrhizae involves several stages. It begins with propagating the arbuscular mycorrhizal isolate MAA-01 in culture pots using a media ratio of 5 kg of sterile soil mixture to 5 kg of sterile manure (1:1). Each culture vessel is then inoculated with up to 40 g of the arbuscular mycorrhizal isolate MAA-01. Maize is planted in these pots as a host plant and maintained for 42 days (6 weeks). After 6 weeks, the culture pots are harvested and air-dried until the water content reaches 10%. The roots of the harvested maize plants are finely chopped and then thoroughly mixed with

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soil that has been sifted through a 2 mm diameter sieve. The resulting arbuscular mycorrhizal inoculant powder is a blend of mycorrhizal spores, hyphae, infected roots, and soil containing mycorrhizae.

The second stage involves the formulation of the improver and arbuscular mycorrhizae by mixing 25% powdered mycorrhizal inoculant with 25% powdered compost, 25% powdered manure, and 25% rice husk charcoal powder as carriers in a volume ratio of 1:1:1:1. The mixture is then blended using a mixer to ensure it forms a homogeneous mass. This mixture is subsequently sieved through a 10-mesh sieve by shaking until a moist powder is achieved. This wet powder is then air-dried for 12 hours. After drying, the arbuscular mycorrhizae breeding powder undergoes testing to determine the number of spores, mycorrhizal colonization, water content, and shelf life. The resulting ameliorant powder, enriched with arbuscular mycorrhizae, is packaged in aluminum foil. This inventive process produces a powder formulation that significantly enhances mycorrhizae, evidenced by 2,500 spores per 20 g of powder, a colonization percentage of 95%, a water content of 5.002% db, and a shelf life of 12 months. The flowchart of the research process is shown in Figure 1 (the process for preparing ameliorant plus mycorrhizal powder as raw material).

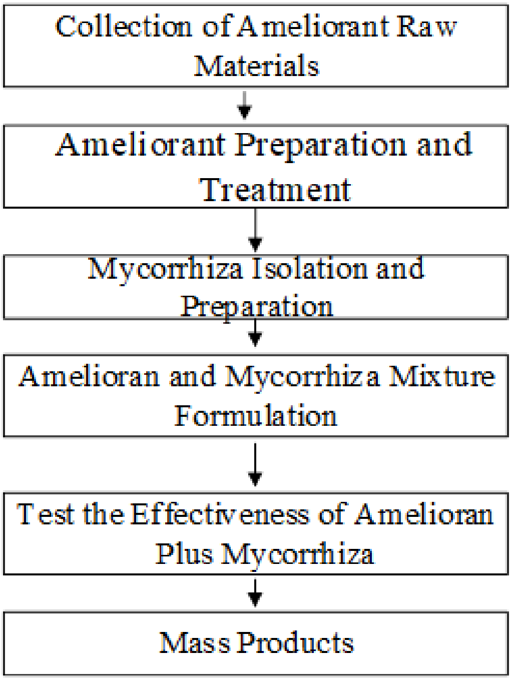

Fig 1 The Process for Preparing Ameliorant Plus Mycorrhizal Powder as Raw Material

C. Data Interpretation

Data were collected through direct observations, documented, and then compared with various research publications from both journals and online databases.

III. RESULTS AND DISCUSSION

Plants require an optimal soil environment for maximum growth and productivity. An important aspect of achieving good soil conditions is improving soil quality. Soil amendments are materials added to enhance soil texture, water retention, and nutrient availability. Additionally, mycorrhizae are microscopic organisms that form a symbiotic relationship with plants, aiding in nutrient and water absorption for enhanced plant growth. Formulations that combine soil improvers with mycorrhizae can offer an innovative solution to boost agricultural productivity on suboptimal lands [21].

This improver plus mycorrhizal formulation is a composition that includes 25% compost powder, 25% cow manure powder, and 25% rice husk charcoal powder, combined with 25% mycorrhizal inoculant powder in a weight-volume (v/v) ratio of 1:1. The mixture is stirred with a mixer until it forms a homogeneous mass. It is then sieved through a 10-mesh sieve by shaking until a moist powder is achieved. This wet powder is air-dried for 12 hours before being used as a soil amendment. Made from local raw materials, this improver is readily available in areas with suboptimal land. It is effective in enhancing soil physical and chemical properties, including water retention, soil structure, and nutrient availability. Additionally, the formulation includes mycorrhizae, which, although underutilized until now, offer significant benefits for plant growth. Mycorrhizae form a symbiotic relationship with plant roots, aiding in the absorption of nutrients, particularly those less soluble in soil.

Therefore, an amendment plus mycorrhizal formulation is essential as a feedstock for high-quality soil amendments to help reduce reliance on synthetic chemical fertilizers and pesticides, which can harm the environment. Mycorrhiza aids plants in nutrient absorption and reduces the risk of groundwater contamination from chemical fertilizer residues. This formulation not only improves soil quality through enhanced water retention and nutrient availability but also strengthens soil structure. This contributes to reducing soil erosion and maintaining long-term soil fertility. The formulation not only boosts current productivity but also enhances agricultural sustainability over the long term. By improving soil fertility, agricultural productivity can continue without dependence on expensive chemical fertilizers, which are sometimes scarce in the market and negatively impact soil quality and the environment.

This invention is divided into six stages to produce an enhancing powder plus mycorrhizae, which serves as a raw material for soil amendments. The manufacturing process is depicted in the flowchart shown in Figure 1.

The process begins with the collection of local raw materials intended for use in breeding. These may include organic materials such as compost, rice husks, cattle manure, or other suitable local substances. These materials are then processed and prepared, which involves a special treatment to ensure that the improver is sterile and free of pathogens that

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could harm plants or mycorrhizae, using an autoclave sterilizer.

Simultaneously, it is necessary to isolate and prepare the mycorrhizae. This step involves cultivating mycorrhizae in pots under optimal conditions to ensure they remain alive and active. The prepared local raw materials and active mycorrhizae are then mixed into a formulation with a weight-volume (v/v) ratio of 1:1.

The completed formulation undergoes testing to confirm its effectiveness in enhancing soil fertility and its safety for use on plants. This testing includes conducting plant growth experiments with various concentrations of the breeding formulation plus mycorrhizae, using local raw materials in pot trials within a greenhouse setting.

Once the formulation is proven to be both effective and safe, the next step is to initiate mass production. This involves scaling up production to meet market demand.

The flowchart provides an overview of the main steps involved in preparing improver and mycorrhizal formulations from local raw materials. It serves as a basic guide for producing mass soil conditioners that can enhance agricultural productivity and promote environmental sustainability.

The following is an example of a laboratory experiment that tests the impact of various concentrations of breeding formulations plus mycorrhizae, made from local raw materials, on plant growth [22].

In this example, we will utilize maize plants as the test species. Maize is commonly employed in plant growth experiments due to its rapid growth rate and responsiveness to environmental stimuli.

The test method was conducted using a randomized block design in a greenhouse with four formulation treatments, namely: A1: 75% cow manure + 25% mycorrhizal biofertilizer, A2: 75% compost + 25% mycorrhizal biofertilizer, A3: 75% rice husk charcoal + 25% mycorrhizal biofertilizer, and A4: 25% cow manure + 25% compost + 25% rice husk charcoal + 25% mycorrhizal biofertilizer. Each treatment was replicated four times, resulting in a total of 16 experimental pots. Each formulation was filtered using a 2-mesh filter, then mixed with sterile soil in a volume ratio (1:1) of 5 kg using a mixer until homogeneous. Subsequently, a maize seed was planted at a depth of 5 cm in each pot. The test plants were maintained for 42 days (6 weeks), with soil moisture levels, temperature, humidity, and light being carefully monitored. Observed parameters included plant height, number of leaves, wet and dry weight of plant roots and shoots, total nutrient concentration of soil available N and P, nutrient uptake of N and P by the plants, number of spores per 100 g of soil, and percentage of colonization of mycorrhizal fungi.

The test results revealed that the ameliorant formulation consisting of 25% rice husk charcoal, 25% compost, 25% cattle manure, and 25% mycorrhizal powder biofertilizer exhibited the most favorable outcomes in terms of plant height (177 cm), leaf count (13.33), wet root biomass (129.23 g), wet shoot biomass (400.40 g), dry root biomass (90.41 g), dry shoot biomass (106.10 g) per plant, available soil phosphorus concentration (62.96 mg/kg), total nitrogen content (1.75 g/kg), nitrogen uptake (44.96 g/kg), phosphorus uptake (4.10 g/kg) by plants, spore count per 100 g of soil (424 spores), and mycorrhizal colonization (80.66%) at the age of 6 years old. These findings were significantly different from those of other ameliorant mixture formulations.

After determining the optimal improver plus mycorrhiza mixture formulation from the test results, the next step involves preparing the powder formulation of the improver plus mycorrhiza by harvesting its culture. The formulation A4, which is a blend of 25% cow manure, 25% compost, 25% rice husk charcoal, and 25% mycorrhizal biofertilizer, is mixed with sterile soil in a volume ratio of 1:1 at 6 days after sowing. Subsequently, the soil in the growing pot is allowed to dry, and then air-dried until the water content reaches 10%. The roots of the harvested maize plants are finely mixed and evenly blended with the potting soil sifted through a 2 mm diameter sieve. The resulting arbuscular mycorrhizal inoculant powder comprises mycorrhizal spores, mycorrhizal hyphae, infected roots, and mixed soil containing mycorrhizae.

A formulation was then created using the specified ingredients and proportions to produce a blended mixture, consisting of: 25% compost powder, 25% cattle manure powder, 25% rice husk charcoal powder, and 25% mycorrhiza culture pot inoculant powder. The mixture was prepared by weighing 500 g of mycorrhizal inoculant and adding 500 g each of compost powder, powdered manure, and rice husk charcoal, maintaining a volume ratio of 1:1:1:1. This blend was thoroughly mixed using a mixer until a homogeneous mass was achieved. Subsequently, the mixture was passed through a 10-mesh sieve by shaking, resulting in a powdered consistency. The powder was then placed in a baking dish and air-dried for 12 hours to achieve a safe moisture content suitable for storage for up to 12 months.

The moisture content of the mycorrhizal biofertilizer dry powder was measured using the heating method [23]. A 5 g sample was placed in a cup with a known weight and then dried in a vacuum oven at 70°C until a constant weight was achieved. Following calculation, the powder was found to have a moisture content of 5.002% db.

The spore number test is conducted through wet sieving (wet sieving and decanting) [24]. Initially, a 100 g sample is soaked in a glass container with 400 ml of water for 15 minutes. Following this, it is agitated with a magnetic stirrer for 6 minutes and then filtered sequentially through grids with diameters of 300 µm, 106 µm, 53 µm, and 38 µm. The resulting filter is rinsed with a hand sprayer until clean. The material retained on the 38 µm filter is transferred to a

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centrifuge tube and mixed with 45 ml of water, then centrifuged at 2000 rpm for 15 minutes. The supernatant is collected, mixed with 25 ml of 50% sugar solution, and centrifuged again at 2000 rpm for 10 minutes [25]. The supernatant is sieved using a 38 µm sieve and then filtered through filter paper in an Erlenmeyer flask. The spores on the filter paper are placed in a Petri dish for counting under a stereomicroscope at 40x magnification. The calculation results indicate an average of 7,500 spores per 100 g of soil.

The calculation of the colonization percentage is conducted using a modified cleaning and staining method [26]. Initially, the roots are washed and cut into approximately ±1 cm pieces, followed by immersion in a 10% KOH solution for 30 minutes in a water bath. After rinsing with tap water to remove residual KOH solution, the roots undergo another washing cycle until clean and are then soaked in 5% HCl for 2 minutes. Subsequently, the roots are deeply immersed in trypan blue lactoglycerol (0.05%) and heated to boiling for approximately 3 minutes. Following this, the excess dye is discarded, and the roots are preserved in bottles containing lactoglycerol 0.05%. The stained roots are observed under a microscope to determine the percentage of infection. The calculation yields an average colonization percentage of 95%. The infection percentage is determined using the formula:

$$= \frac{number\ of\ infected\ roots}{the\ total\ number\ of\ root\ pieces\ observed}\ x\ 100\%$$

The shelf life of the enhanced powder plus arbuscular mycorrhizal biofertilizer was estimated by storing the arbuscular mycorrhizal biofertilizer powder in aluminum foil containers at room temperature. Observations of changes in the number of spores were conducted weekly. The results indicated a decline in the number of spores by the 54th week of storage. This decrease could be attributed to the obligate symbiotic nature of arbuscular mycorrhiza, which relies on its host plant for survival. It is suspected that the decline in spore count at week 54 occurred due to the absence of a carbon supply, resulting from the lack of a host plant.

The ultimate objective of this invention has been achieved by producing a powder formulation of arbuscular mycorrhizal enhancer fertilizer that contains 2500 spores per 20 g of powder, boasts a colonization percentage of 95%, maintains a water content of 5.002% % db, and possesses a shelf life of 12 months.

This discovery offers several advantages, notably in its simple production process for creating a powder builder formulation plus mycorrhizal biofertilizer, which requires only basic technology such as a sieve and dryer. The drying tool utilized, a baking tray placed outdoors, is also straightforward, making it applicable to farmer-scale industries. Additionally, the raw materials for producing powdered mycorrhizal biofertilizers are readily available, sourced from abundant plant residues accessible throughout the year. These materials are environmentally friendly and contribute to enhancing soil fertility, promoting plant growth, and boosting yields, particularly in challenging conditions on suboptimal lands.

Through the research process, a powder blend of improver and mycorrhizal inoculant was developed, boasting 2500 spores per 20 g of powder, a colonization rate of 95%, a water content of 5.002%, and a shelf life of 12 months, as outlined in Table 1.

Table 1. Results of the Ameliorant Powder Formulation

No	Type of test	Test results
1	Number of spores	2500 spores/20 g powder
2	% cologne	95%
3	Water level	5.002 % db
4	Duration	12 months

IV. CONCLUSION

The research findings demonstrate the process of formulating an ameliorant blended with indigenous arbuscular mycorrhiza, using the *Glomus mosseae* isolate MAA-01, along with a carrier derived from plant residues such as compost, cow manure, and rice husk charcoal. The culture substrate for the *G. mosseae* isolate MAA-01 consisted of 25% compost powder, 25% manure powder, and 25% rice husk charcoal powder, resulting in a spore count of 2500 per 20 g of powder, a colonization rate of 95%, a water content of 5.002 % db, and a shelf life of 12 months. This formulation of ameliorant plus mycorrhiza powder, crafted from locally sourced raw materials, yields a high-quality product with a substantial spore count and colonization rate. Its simplicity and ease of production make it suitable for farmers. Laboratory tests confirm its efficacy as a soil amendment capable of enhancing soil fertility, promoting growth, and increasing yields on suboptimal land.

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