Multiplication Arbuscular Mycorrhizal Fungi in Corn (*Zea mays* L.) with Pots Culture at Greenhouse

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Abstract. This study was multiplicated by Arbuscular Mycorrhizal Fungi (AMF) indigenous in corn with pots culture at the greenhouse. The research will be conducted from August 2019 to October 2019 in Greenhouse, Laboratory of Microbiology, Mataram University, Indonesia. This research aims to determine the influence of AMF in dry land and the application of fertilizer concentration. This research was conducted isolate exploration in four villages at the Pujut Central district, Lombok, Indonesia i.e. Mertak, Sukadana, Kuta, and Sengkol Village. This research is an experimental study with a completely randomized factorial design with two factors i.e the AMF isolate type and the concentration of Johnson's nutrient solution. The first factor with the level without AMF Isolates, Isolate 1, Isolate 2, and Isolate 3. While the second factor is the Johnson nutrient concentration i.e 50 % and 75 % solution. The results showed that were differences in growth such as crop height and the number of leaves where Isolate 1, gave the highest growth and number of leaves. The identification was obtained the Isolate 1 showed highest spore's density and root infections is Isolate 1 with a spherical shape.

Keyword: Environmentally friendly, increase productivity in dry land, microbial fertilizer, phosphorus uptake

1 Introduction

Arbuscular mycorrhizal fungi (AMF), phylum Glomeromycota, are obligate root symbionts that are present in most terrestrial ecosystems and establish a mutualistic symbiosis with several plant species around the world [1]. They produce structures inside plant roots (e.g.,

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arbuscules), thus having an important role in plant mineral nutrition, (e.g., P-uptake, N-uptake and micronutrients- uptake) and water absorption [2, 3], resulting in increased plant growth, resistance, and tolerance to abiotic and biotic stresses, such as soil-borne pathogens and drought [4].

Plants continuously interact with other microorganisms present in their environment [5, 6]. Moreover, they are able to establish mutually beneficial associations with some of these microorganisms present in the rhizosphere. One of the most well studied benefificial plant microorganism associations is that established with certain soil fungi known as AMF [2]. Interestingly, the vast majority of land plants, including most agricultural crop species, are able to establish AM symbiosis [2]. It positively affects plant growth and provides tolerance against biotic and abiotic stresses [7]. AMF is known as soil fungi in the rhizosphere area. AMF was a mutualism fungus with plants root i.e. Ectomycorrhizal and Endomikoriza [2]. Besides that, AMF work in poor soils to uptake and transport nutrients (macro and micro), although mycorrhizal association role related to carbon flux is less defined [8, 9]. AMF may live symbiotically on the roots of approximately 80 % of different plant types [10, 11].

AMF provided many benefits, especially on marginal land, i.e., benefits for ecosystems, crops, and humans. That application for the ecosystem increased to the nutrient cycle, improved the soil structure, and channeling carbohydrates from plant roots to other soil organisms. Furthermore, the advantages for plants increased the absorption of nutrients, mainly Myxedema may emit phosphatase and acid-organic acids. [11], crop resistance and tolerance to the abiotics and biotics such as dryness and soil pathogens. Based on the role, AMF is the potential to develop as a biological fertilizer for grew and productivity. The AMF multiplication developed as a biological fertilizer [12, 13]. AMF multiplication by pots culture and a single culture in laboratory [14, 15]. The reproduction of spores by pot culture used as a source of spores, then sterilized and used as a sterile incarnated source for the development and reproduction of the AMF of axiom in vitro cultures [16, 17].

The AMF multiplication factor, i.e., as compatible hosts, places to grow, and the environment. The no less important is the high power of infection and effectiveness, colonization of host roots rapidly and produce many spores [2, 16]. AMF did not choose a specific host, all plants are potentially infected, but the degree of infectivity and effectiveness differs from each host association with AMF. The AMF added to infected and colonized in root plant, type of host plant were *Sorghum bicolor* L.*, Zea Mays* L.,*Panicum maximum* Jacq.*, Paspalum notatum* L.*, Arachis hypogaea* L.*,* and *Pueraria javanica* Roxb. Benth. [17, 18] with various planting media included soil, sand, peat, clay and zeolite [13]. The selection of Inoculum and host plants is the key to success in the cooperation system of both organisms.

The factors of AMF multiplication was a plant variety, AMF type, and fertilizer application [2, 18]. Dosage Phosphate influenced by the form of Phosphate used, inorganic, as well as organic or insoluble and insoluble in soil [2, 15]. Generally, fertilizers used in AMF spores production was nutrient solutions with low Phosphate levels.

This study aims to determine the influence of the isolates and concentrations of Johnson's nutrients against i) plant growth, ii) soil chemical properties, and iii) spores' density.

2 Methods

This research conducted by a Completely Randomized Factorial Design (CRFD) with three repeated. The first factor with the level without AMF Isolates, Isolate 1, Isolate 2, and Isolate 3. While the second factor is the Johnson nutrient concentration i.e 50 % and 75 % solution. This research was conducted in Faculty of Agriculture, University of Nahdlatul

Wathan, Laboratory of West Nusa Tenggara Assessment Institute for Agricultural Technology and Microbiology Laboratory, Faculty of Agriculture, University of Mataram, Indonesia. That research was from August 2019 to October 2019.

The characteristics three types of isolat based by [19]:

2.2.1 Glomus sp.

Spores reddish brown, spores slightly rounded form. The spore wall consists of two layers namely layer A and layer B. Layer A is the first layer of thin membrane. Layer B is the second layer. *Glomus* spp. morphology of microscopy. Shown in Figure 1b

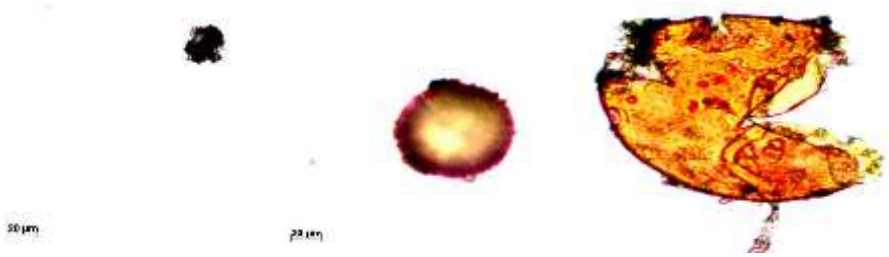
2.2.2 Gigaspora sp.

The sprouts produced from the spore wall, have spores such as thorns and have a single spore, a globus-shaped, ovoid or irregular, and a yellowish wall. Its microscopic morphology can be seen in Figure 1a

2.2.3 *Acaulospora* sp.

Spores colored brownish-yellow. The spores' developmental process seems to be from Hyfa but it is not so. First there is a hypha whose tip is enlarged like a spore called hyphae terminus. Show in Figure 1c

Gigaspora sp. *Glomus* sp. *Acalauspora* sp.



a b c

Fig. 1. Type of mycorrhiza

Data Analysis used Analysis of Variance (ANOVA). The conclusion decides based on the F test shown the significance of data and application, then the continued analysis with Tukey's HSD (honestly significant difference) test. The observational variables. The observational variables consist of two parts, i.e., plant growth included high crop, a broad index of leaves, leaf characterization, spore density and mycorrhizal diversity.

3 Results and discussions

3.1 Plant height (cm)

This study observed two times measurements to plants height followed the stages of corn growth. The plant height observed at 14 days after planting (DAP) and 28 DAP. At 14 DAP Corn has entered the V3 to V5 (the number of leaves opened was three strands to five strands), This phase occurs at 10 d to 18 d after germination, and the seminal root grow stopped, furthermore the primary and lateral of the root to be active on the grows below ground level. Ground temperature affected the growing point, while the low temperature would slow out the leaves, increased the number of leaves, and delayed the formation of male flowers [20].

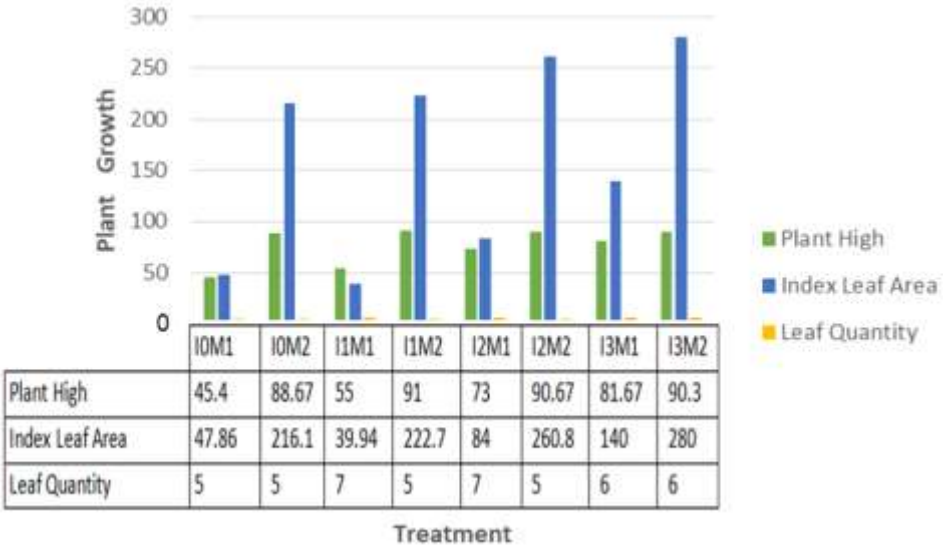
While at 28 DAP of the V6 to V10 leaves was opened in six strands to ten strands. This phase takes place at the time of the plant between 18 d to 35 d after germination. The growth point is already above ground, the development of roots and quick spread on the ground, and the stem enlargement increased rapidly. In this phase will be male flowers (tassel) and the development of cob begins. Plants begin to absorb the nutrients in more quantities. Therefore, fertilization in this phase is necessary to provide nutrient needs for plants.

The results of diversity analysis show there is a noticeable difference in the height of the plant at the age of 28 DAP that is seen in isolates factors, dosing factors and Interaction both.

3.2 Leaf area index

Leaf area index (LAI) indicated crop potentials in photosynthesis. It can directly affect growth and development of plants. The leaves wider in a plant thus maximum absorption of light. According to Gardner and Pearce [20], The wide index range of leaves that are optimal for cultivation plants is from a value of three to a value of five. Based on Table 2, the treatment of phosphate rocks at a dose of 50 % from the recommendation with the application of biological fertilizers as much as two times tends to give better results to the broad index of leaves compared with control and other treatments. Nevertheless, all treatments do not give a noticeable effect on the broad index of leaves.

Plants require large quantities of P, which they obtain as phosphate (Pi) from the solution phase of the soil. While many soils have a high P content, it occurs mostly as complex organic or inorganic forms that are not directly accessible to the plant, and Pi levels in the soil solution are below 10 µM (21). The addition of phosphate nutrients in the corn plant did not encourage an increased increase in the area of the leaf because the phosphate nutrient was not a broad index boundary of the leave. That's factors affected the magnitude of the broad index of leaves include the spacing and supply of nitrogen nutrients. There is no difference in spacing and fertilizer doses of nitrogen nutrient providers used by all the treatments in this experiment.



	I0M1	I0M2	I1M1	I1M2	I2M1	I2M2	I3M1	I3M2
Plant High	45.4	88.67	55	91	73	90.67	81.67	90.3
Index Leaf Area	47.86	216.1	39.94	222.7	84	260.8	140	280
Leaf Quantity	5	5	7	5	7	5	6	6

Fig 2. Influence type of isolate and concentration of Johnson nutrie solution on plant growth

3.3 Soil analysis

The distribution of Mycorrhiza is influenced by many factors, such as soil type and structure, nutrient elements Phosphate and Nitrogen in soil, water, pH, and soil temperature. the results was content analysis and soil conditions at all three sampling locations that can be seen in Table 1.

Table 1. Soil analysis

Soil analysis	**Nutrient value**
The water	8.39
pH	6.83
N (%)	0.30
P (mg kg^{-1})	15.30

The physical properties of other soils are pH. That showed pH is approaching neutral and the analysis of physical and chemical properties the soil aimed to determine the existence of mycosis. The state of the soil is very high in population, colonization and Mycorrhiza.

Based on the results of chemical analysis of soil is known that soil pH found in research area classified as neutral. The content of elements N and P availability of very low conditions.

Mycorrhiza is influenced by several environmental factors such as light, temperature, groundwater content, soil pH, organic matter, and heavy metals and other elements [21]. From the results of soil analysis, have a soil pH content of H_2O (1:1.5) 6.83 (neutral), P Bray-1 15.3 mg L^{-1} (very low), N total 0.30 % (low).

The number of spores and Mycorrhiza type is closely related to soil chemical conditions. In the range of pH 6.85, The number and type of Mycorrhiza is increasing [22]. Generally mycorrhizal is more resistant to soil pH changes. However, the pH changes in the soil rhizosphere have a direct impact on the solubility of Al in the soil. The more acidic soil

pH, the rate Al in the soil is increasing, and this has an impact on the decrease in the amount and type of Myoriza. This is because Al is able to inhibit root development. As a result, plants easily experience water, stunted growth, and biomass and low productivity [23].

At soil pH 4.5 to 8.0, P, and C-organic increases, then the number and type of Mycorrhiza will increase. This is because pH determines the ease of nutrient absorbed plants including the element P, where P serves for cell division, assists in the transfer of energy in metabolic activities, resulting in good plant growth, and ultimately assists the development of Mycorrhizal. C-Organic can also guarantee the occurrence of mineralization which results can provide nutrients for symbiotic mybiosis with plants, besides organic matter can induce the growth of hypha mycorrhizal [22].

Based on the results of analysis in the Laboratory (Table 1) on the soilchemical properties indicates the level of soil (pH) of the average land reaches 6.06. Soil chemical properties are known to affect the ability of mycolic fungi associated with plants strongly, this is in accordance with the statement Prihastuti [23], Mycorrhizal can live well in sour soil pH and able to produce organic acids that Freed P fixation.

Phosphor content in soil is known to affect the variety of colonization of microchemical fungi in plant roots. The results of soil analysis against P are available in the soil, indicating very low criteria of 0.30 mg L^{-1}. Soil that contains a high element P is often associated with the decline of Mycorrhizal colonization. The formation of a symbiosis of arbuscular mycosis fungi reaches a maximum if the level P in the soil is not greater than 50 mg L^{-1} [15].

Soils containing high nitrogen and phosphate then the amount of his mycorrhiza is also high. This is because according to Setiadi [24] wherein soil nitrogen comes from soil organic matter, binding by microorganisms from air nitrogen, fertilizer, and rainwater, so that the nitrogen content in the soil is strongly correlated with the amount of Mycorrhizal found. Similarly, the content of phosphate, in which the research was obtained an average value of 0.26. This is because phosphate is a nutrient that is very influential in the presence of Mycophoriza, where phosphate is a macronutrient for plants so that the existence of Mycorrhiza is very helpful in plant growth where hypha Mycorrhizal assists in the absorption of phosphate in the soil. Phosphate that has been absorbed by the external Hypha will soon be converted into a polyphosphate compound and transferred into the internal Hyfa and Arbuskul. In the Arbuskula, polyphosphate compounds are broken down into organic phosphate which are then released into host plant cells.

High or very low moisture and groundwater content are also less good for Mycorrhiza development. Mikoriza develops in humidity and moisture content that is stable, not too high and not too low. When the moisture content and humidity is high or excessive can cause anaerobic conditions that inhibit the development of mycodone because all microform fungi are aerated [25]. Meanwhile, low groundwater content causes dryland conditions. Dryland is very supportive for the development of Mycorrhizal, where the availability of low nutrient on the condition of dry land will optimize the development of Hypha mycorrhizal [26].

Further, Setiadi [24] reported that Mycorrhiza was instrumental in increasing crop tolerance to critical land conditions, drought and heavy metals. The colonization of plant roots with Mycorrhizae may affect communities associated with direct and indirect roots. Direct interactions include the provision of energy-rich carbon compounds, changes in mycorrhizosphere pH, nutritional competitions, and fungal exudation from inhibition or stimulation of compounds. Direct interaction can also occur in the form of mycorrhizal effect on the growth of host crops, the results of root exudation and improvement of soil structure.

3.4 Density and diversity of 'Arbuscular Mycorrhiza Fungi' spore

Diversity of AM fungi with host plant which impart significant impression on plant nutritional status, morphology, genetic expression and symbiotic efficiency in symbiotic relation [27–29].

Mycorrhizal spores' populations i.e., Glomus was high population also thought to be due to environmental conditions that are more suitable, optimal, and compatible in supporting the growth and development of spores. Supported again by the possibility of the absence of antagonistic mushrooms that inhibit mycorrhiza sporulation. Other factors, environmental differences from soil, nutrients, altitude, rainfall, light on both soil examples, allow the difference of spore's density.

In areas that have higher nutrient elements and more loose soil structure found a diversity of type and the number is relatively lower. This is because when the nutrients are insufficient condition, the root of the plant can act as a nutrient absorbent organ so that the plant accumulates nutrient elements in high quantities. The condition will cause a negative response to Mycorriza colonization. This information was emphasized by Subiksa [30], that the fertile media and the increasing P elements in the soil can decrease the activity and infection of mycosis, and even the population will be reduced due to some death.

The Genus Glomus, Acaulospora, and Gigaspora found in the rooting (the rhizosphere) of corn plants allegedly have the suitability of environmental conditions for spores development. This suggests that the genus Glomus and the genus Acaulospora can thrive. According to Brundrett et al. [31], this type of soil and host plant type affects the genus Mycorrhizal found and its effectiveness towards host crops. The dispersion pattern of each MVA mushroom genus is different. Certain genera have a very widespread and a genus of limited distribution. The genus known to have the most widespread dispersion is the genus Glomus.

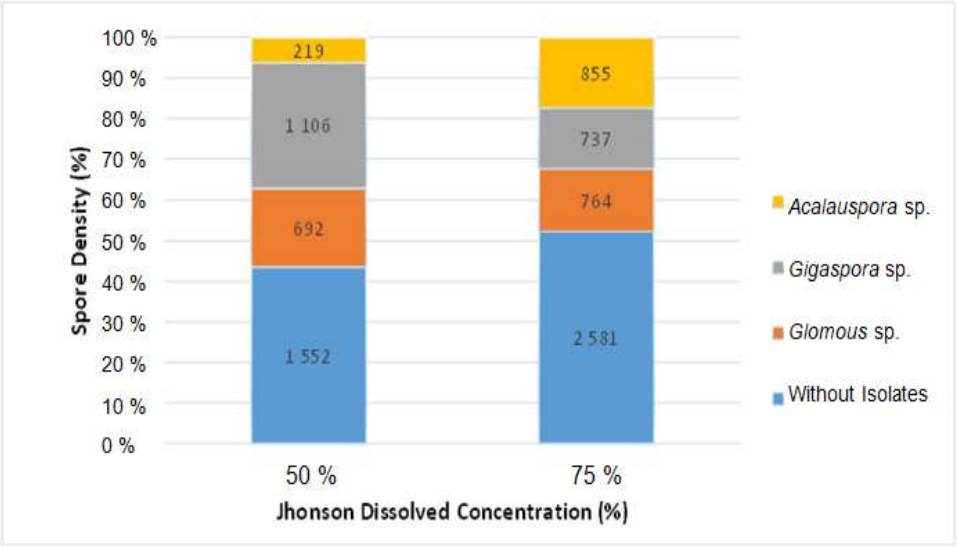


Fig.3. Density of spore inoculation treatment of AMF and Johnson nutrien solution

The Figure 3 above, shows that changes in concentration of the Johnson nutrien solution affect the number of spores. A further 5 % BNJ test was carried out which showed that the concentration of the Johnson nutrient solution 50 % produced the highest number of Gigaspora spores of the three types of spore inoculated. The treatment with high nutrient

concentration found a lower number of spores. This is presumably because the nutrients are in sufficient condition so that the roots of the plant can act as a nutrient absorbing organ. Plant will accumulate nutrien elemen in high amounts. This condition will cause negative response to colonization. This information is emphasized In the arbuscular mycorrhizal (AM) symbiosis the reciprocal exchange of nutrients results in a nutritional benefit for both symbionts. The fungus acquires carbon from plant and the plant obtains mineral nutrients from the fungus. While there is evidence for the transfer of phosphorus (P), nitrogen, zinc and copper, current data suggest that P is transferred in the highest quantities and that symbiotic P transfer occurs in the vast majority of AM symbioses [2]. To strongly corelated at change P with colocalized in AM Fungi [32]. Expression of Pt4 and transfer of phosphate are preconditions to establish successful symbiotic relationship and arbuscule formation [33].

4 Conclusion

From the results of the research and the discussion can be withdrawn several conclusions as follows

i. Isolate and dosage factors affect the growth of corn crops such as high crop age 14 DAP and 28 DAP, and Broadleaf index
ii. pH soil, P nutrien content affects the number of spores in the soil, so the higher the concentration of P nutrient, the number of spores in the soil will be low
iii. There was an increse in the number of spores in the soil with the most types *Glomus*, *Gigaspora, Acalauspora*

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