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Research Article

Propagation Techniques of Mycorrhizal Bio-fertilizer with Different Types of Mycorrhiza Inoculant and Host Plant in Entisol Aceh

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Abstract

Background and Objective: This study aimed to produce of mycorrhizal bio-fertilizer in entisol Aceh and to reduce farmer's dependence on inorganic fertilizers. Especially, in coastal areas of Lampuuk Aceh Besar with order entisol soil has been constrained to crop management. The soil is not only low-content of nitrogen, phosphorous and potassium but also not optimally absorbed by plants. Besides, the availability of water to be absorbed by plants in entisol also limited. Strategies to overcome these constraints, we need to produced mycorrhizal bio-fertilizer by different types of mycorrhizal inoculants and host plants and applied it to increase the productivity of entisol and crop. Propagation of mycorrhizal bio-fertilizer was carried out by using three types of mycorrhizal inoculants consisted of *Glomus mosseae*, *Gigaspora* sp., mixing *Glomus mosseae* and *Gigaspora* sp. on the host plants of maize and white sorghum, respectively. **Methodology:** This experiment was arranged in a non-factorial randomized block design with three replications. The parameters observed included the quality of mycorrhizal bio-fertilizer following of mycorrhizal infection on host plant, spore population, root fresh weight and root dry weight. **Results:** The results showed that the types of mycorrhizal inoculant and host plant affected the quality of mycorrhizal bio-fertilizer. **Conclusion:** The inoculation of mycorrhizal with the starter *Glomus mosseae*+*Gigaspora* sp. and the host plant of corn gave the best results and have the potential for propagation techniques of the mycorrhizal bio-fertilizer on entisol Aceh. The alternatives can also be done using a starter *Gigaspora* sp. and the host plants of white sorghum.

Key words: Mycorrhizal, bio-fertilizer, production, *Glomus masseae*, *Gigaspora* sp., entisol

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mycorrhizal is a mutualistic symbiosis form between fungi and plant roots. Almost all types of plants are the form symbiotic with mycorrhizal fungi and play a role in improving the tolerance of crops to the conditions of marginal land, drought, lack of nutrients and contaminated land (Chauhan *et al*., 2011; Langer *et al*., 2010; Brundrett *et al*., 1996. Various study on mycorrhizal have been conducted but it is still a little bit of study on techniques to produce it. Simanungkalit (2003) stated effort of producing mycorrhizal inoculants on a large scale was still rare and difficult. Therefore, it is necessary for further studies in an effort to maximize production potential of mycorrhizal bio-fertilizer.

Mycorrhizal bio-fertilizer inoculum can be produced through propagation of marginal land to increase fertility. Generally, production of bio-fertilizer of Arbuscular Mycorrhizal Fungi (AMF) or propagation of inoculum can be conducted in a clean and environmental protected and usually takes 75 days by using the pot culture method. Some plants that can be used as a host plant are the leek (*Allium porrum* L.), Sudan grass (*Sorghum bicolor* (L.) Moench), Bahia grass (*Paspalum notatum* Flugge), maize (*Zea mays* L.) and Puero (*Pueraria javanica* L.). Selection of appropriate host plants, need to be considered due to the interaction between the host plant, types of AMF, media composition and climate for growth (Falah 2011; Struble and Skipper, 1988).

Maintenance phase of the plant was conducted to host approximately 2 months after planting. Plants should be placed in enough sunlight. Regular watering is not necessary but it is enough to keep moisture in planting medium. Fertilization also performed sufficiently by choosing a liquid fertilizer that contains elements of P lower with the main nitrogen fertilizer (Chauhan *et al*., 2011; Matsubara *et al*., 1996).

Stressing is a stage in the form of an attempt to inhibit or suppressing the growth of the host plant with certain conditions. The goal of stressing to spur the AMF and to form of spores. Spores this will then be harvested and be a source of inoculum (starter of mycorrhizal) (Falah, 2011; Jumini *et al*., 2014; Douds *et al*., 2010).

Topping or cutting the top of host plants was conducted by leaving only its rootstock±¼. In such an unfortunate circumstances AMF will form resistant structures in the form of spores to survive (Falah 2011; Norland, 1993). Harvesting of mycorrhizal bio-fertilizer on host plants can be carried out after experiencing stressing host plant due to 1 month or ±3 months after the initial planting and carried out by dismantling the host plant and take part of roots. Then root cutting into small pieces (±0.5 cm) and mixed with their cropping media (Jumini *et al*., 2014; Syafruddin *et al*., 2010).

Plants were given inoculum AMF have higher productivity and resistance to attack by pathogen than plants that grow without being given inoculant AMF. Mycorrhizal infection can enhance plant growth and their ability to utilize available nutrients in soil, especially the elements of P, Ca, N, Cu, Mn, K and Mg. Mycorrhizal colonization of the roots can expand the area of root uptake in the presence of external hyphae grow and develop through the roots (Cameron, 2010; Feldmann and Idczak, 1992; Adelman and Morton, 1986).

Especially in Aceh chili and other vegetables are generally cultivated in andisol and entisol. Furthermore, in the coastal areas such as Lampuuk, Peukan Bada, Aceh Besar cultivation of chili and other vegetables was largely cultivated on entisol. The characteristics entisol were low-content of nitrogen, phosphorous and potassium, soil organic matter and CEC (Syafruddin *et al*., 2010; Hardjowigeno, 2008).

Constraints in entisol not only the low-content of nutrients but also low water holding capacity that cannot be utilized by plants. The improvement of soil fertility by using bio-fertilizers needs to be done. One well-known bio-fertilizers are mycorrhizae. Mycorrhizae helps the absorption of nutrients that plants need particular elements such as nitrogen, phosphorus and potassium. While, the plant can be provided carbon element needed by the fungus for survival (Medina and Azcon, 2010; Smith and Read, 1997). Mycorrhizae virtually present in all host plants both crops, horticulture and plantation which can act as bio-fertilizer, bio-protector and bio-regulator that make it as environmentally friendly biological agents (Liu *et al*., 2014; Rosliani and Sumarni, 2009; Simanungkalit, 2006).

The utilization of mycorrhizal bio-fertilizer of *Glomus mosseae* and *Gigaspora* sp., for chili and other vegetables can improved growth and their production. This is due to the ability of roots to absorb nutrients and to increases of crop protection from pathogens, resistant to drought and other extreme conditions (Purnomo *et al*., 2008; Agustin *et al*., 2010; Abbott and Gazey, 1994) and help the absorption of phosphate and nitrogen (Prasetya and Anderson, 2011; Musfal, 2010; Simanungkalit, 1993). The AMF influenced of increasing growth and yield of soybean production and soil phosphorus levels on interactions between vesicular-arbuscular mycorrhizae and rhizobium in soybeans (Zuhry and Puspita, 2008; Asimi *et al*., 1980). Boddington and Dodd (2000) reported that FMA different genus produce a different response to the management when associated with the cultivation of plants and agricultural practices in the tropics.

Thus, the aimed of this research to produce mycorrhiza biological fertilizer as bio-fertilizer and bio-protector with different mycorrhizal inoculant and host plants in entisol Aceh that could be used to increase production of crop and soil quality.

MATERIALS AND METHODS

Soil preparation, starter mycorrhiza, host plants and propagation techniques: Propagation techniques of mycorrhiza was conducted using pot culture method by planting corn seeds in plastic pots size of 15 kg soils. Preparation and sterilization of media in the early stages by using autoclave at 120°C for 20 min. Corn seed germination were planted on the media. The next step was to put the starter mycorrhiza roots form mycorrhiza/AMF spores around the roots as much as 0.5-1 g. Mixed starter containing a minimum of 10-20 spores. After that plants were fertilized with N, P, K according to recommendation. Maintenance phase was conducted until the plants 2 months after planting. Then stressing the plant was designed to create the structure of the soil in the form of spores. Spores were harvesting and this is a source of inoculum (starter mycorrhizal). Harvesting was conducted by dismantling the host plant and take part of roots. Roots were cut into small pieces (0.5 cm) and mixed in planting media. Mycorrhiza bio-fertilizer applied as a biological fertilizer (bio-fertilizer) and bio protector to the field with the recommended dose of 10 g/plant for chili and other vegetables.

Design of experiments: Experiments were laid out in a randomized block design with the treatment as follows: *Glomus mosseae* inoculant and corn host plant, *Gigaspora* sp., inoculant and corn host plant, *Glomus mosseae*+*Gigaspora* sp., inoculant and corn host plants, *Glomus mosseae* inoculant and white sorghum host plants, *Gigaspora* sp., inoculant and white sorghum host plants, *Glomus mosseae*+*Gigaspora* sp., inoculant and white sorghum host plants. The experiment was arranged with three replications and have 18 trial unit. Each pot culture was added by 10 g mycorrhizal inoculant in 2 kg soil.

Parameters observed: The 2.5 months after planting the plants were harvested and collected the root to inspect the level of mycorrhizal root infection on the host plant. Then calculating the spore population, root fresh weight and root dry weight were conducted. Infection level of AMF on the roots of host plants viewed through methods by Langer *et al*. (2010) and staining of roots conducted through methods by Kormanik and McGraw (1982). Population of spores calculated with the method by Brundrett *et al*. (1996).

Statistical analysis: Data were analyzed by analysis of variance (ANOVA) technique. The significance of treatment effect was performed by using the F-test. The significant difference between treatment means the Tukey's HSD test at level 5% ($p<0.05$; HSD test).

RESULTS

Soil characteristics: Based on the results of the analysis of entisol Aceh (Table 1) that for using propagation of mycorrhizal bio-fertilizer were required the addition of nutrients mainly nitrogen, phosphorous and potassium. This is necessary to stimulate the vegetative growth of the host plant, considering the very low nutrient in entisol Aceh. However, the pH value of the soil is suitable to the criteria for cultivating of the plant. Generally, after the addition of nitrogen, phosphorous and potassium fertilizer as recommended could be stimulated vegetative growth of plant.

Level of mycorrhizal infection on host plants: Results of analysis of variance (ANOVA) showed that the level of mycorrhizal infection of host plants affected by mycorrhizal starter and the type of host plant. The highest mycorrhizal infection rate found in the type of starter mycorrhizal *Glomus mosseae*+*Gigaspora* sp., inoculates on corn's host plants but was not different with *Glomus mosseae* inoculates+corn, *Glomus mosseae* inoculates+white sorghum, *Glomus mosseae*+*Gigaspora* sp., inoculates + white sorghum (Table 2). While, the lowest result in the encounter on *Gigaspora* sp., inoculates+white sorghum and *Gigaspora* sp., inoculates+corn.

Table 1: Characteristics of soil experiment (entisol)

Parameter value	Method
pH H_2O 6.70	pH 1: 2.5
pH KCl 6.60	pH 1: 1.25
C-organic (%) 0.61	Walkley and black
N total (%) 0.07	Kjeldahl
P av (ppm) 2.31	Bray II
K (me 100 g^{-1}) 0.18	NH_4OAc pH 7
KTK (me 100 g^{-1}) 9.50	NH_4OAc pH 7
Texture sandy loam	Pipette
Sand 67.58%	
Silt 22.42%	
Clay 10.00%	

Sources: Syafruddin and Efendi (2014)

Table 2: Average of mycorrhizal root infection

Treatment (starter mycorrhizal, host plant)	Percentage infection of mycorrhiza (%)
Glomus mosseae inoculates and corn	71.33[ab]
Gigaspora sp., inoculates and corn	66.00[a]
Glomus mosseae+*Gigaspora* sp., inoculates and corn	76.00[b]
Glomus mosseae inoculates and white sorghum	72.67[b]
Gigaspora sp., inoculates and white sorghum	66.67[a]
Glomus mosseae+*Gigaspora* sp., inoculates and white sorghum	71.33[ab]
Tukey's HSD test (p<0.05)	5.69

Value followed by the same letter, the same columns is not significantly different at Tukey's HSD test (p<0.05)

Table 3: Average of No. of AMF spores (per 100 g soil)

Treatment (starter mycorrhizal, host plant)	No. of spores (g)
Glomus mosseae inoculum and corn	155.00[a]
Gigaspora sp., inoculum and corn	381.00[b]
Glomus mosseae+*Gigaspora* sp., inoculum and corn	637.00[e]
Glomus mosseae inoculum and white sorghum	551.67[d]
Gigaspora sp., inoculum and white sorghum	635.00[e]
Glomus mosseae+*Gigaspora* sp., inoculum and white sorghum	533.33[d]
Tukey's HSD test (p<0.05)	20.56

Value followed by the same letter, the same columns is not significantly different at Tukey's HSD test (p<0.05)

Table 4: Average of wet weight of root

Treatment (starter mycorrhizal, host plant)	Fresh weight of root (g)
Glomus mosseae inoculum and corn	12.94[a]
Gigaspora sp., inoculum and corn	24.94[b]
Glomus mosseae+*Gigaspora* sp., inoculum and corn	39.37[e]
Glomus mosseae inoculum and white sorghum	38.73[e]
Gigaspora sp., inoculum and white sorghum	39.74[e]
Glomus mosseae+*Gigaspora* sp., inoculum and white sorghum	36.39[d]
Tukey's HSD test (p<0.05)	01.21

Value followed by the same letter, the same columns is not significantly different at Tukey's HSD test (p<0.05)

Table 5: Average of dry weight of root

Treatment (starter mycorrhizal, host plant)	Dry weight of root (g)
Glomus mosseae inoculum and corn	6.34[a]
Gigaspora sp., inoculum and corn	12.54[b]
Glomus mosseae+*Gigaspora* sp. and corn	19.91[e]
Glomus mosseae inoculum and white sorghum	19.25[de]
Gigaspora sp., inoculum and white sorghum	19.92[e]
Glomus mosseae+*Gigaspora* sp., inoculum and white sorghum	18.67[d]
Tukey's HSD test (p<0.05)	0.88

Value followed by the same letter, the same columns is not significantly different at Tukey's HSD test (p<0.05)

Spore population: Results from analysis of variance (ANOVA) performed that the type of mycorrhizal inoculant and host plants significantly affected the spore population in entisol Aceh. The highest spore population was found in type of mycorrhizal *Glomus mosseae*+*Gigaspora* sp., inoculum+corn and theses result was not different with *Glomus mosseae*+*Gigaspora* sp., inoculum+ hite sorghum. The lowest result was found on *Glomus mosseae* inoculum+corn (Table 3).

Root fresh weight: Results of analysis of variance (ANOVA) assumed that the root fresh weight was influenced by the type of starter mycorrhizal and host plants. The highest root fresh weight was found in the type of mycorrhizal with starter *Gigaspora* sp., inoculates and on host plants of white sorghum and was not different with *Glomus mosseae*+*Gigaspora* sp., inoculum+corn and *Glomus mosseae* inoculum+white sorghum, respectively. The lowest result was found on *Glomus mosseae* inoculum+corn.

Root dry weight: Results of analysis of variance (ANOVA) described that the root dry weight was influenced by the type of starter mycorrhizal and host plants are used. The highest root fresh weight was found in the mycorrhiza starter *Gigaspora* sp., inoculates and host plant of white sorghum. These result did not differ by *Glomus mosseae*+*Gigaspora* sp., inoculates, corn and *Glomus mosseae* inoculates+white sorghum (Table 5). The lowest result of root dry weight was found on *Glomus mosseae* inoculates+corn.

DISCUSSION

Utilization of entisol for media propagating of mycorrhizal bio-fertilizer to supply inputs of fertilizer nitrogen, phosphorous and potassium as recommended by the type of host plant. Generally, entisol is considered as a young soil, beginning a new level in the development. The soil has large-porosity and aeration, fast-permeability, low-water holding capacity, additionally the soil has low content of nitrogen, phosphorous and potassium (Table 1). Besides low-CEC and base saturation due to the limited availability of organic material. There was no other horizon identified except epipedon ochric, albic or histic (Hardjowigeno, 2008).

The main limiting nutrient in Entisol are nitrogen, phosphorus and potassium. They are not available sufficiently. In propagation techniques of mycorrhizal bio-fertilizer, we need to supply those fertilizers in order to produce root biomass optimally. As an immature soil, properties of entisol did not unlike the Inceptisols in crop management, especially to increase the biomass of plants including root biomass weight (Syafruddin and Efendi, 2014).

Content of low-organic matter in entisols is favorable for the developing of the AMF and really potential to supply AMF naturally (Pal *et al*., 2013; Prasetya and Anderson, 2011). One attempt to improve the absorption of phosphorus and nitrogen in entisol can be conducted through a symbiosis between plants with mycorrhizal fungi. Hypha of mycorrhizal fungi play an important role in improving N and P uptake by expanding the area of the plant root system, so it can be used to absorb residue P which accumulated in the soil. The FMA

also is able to increase nodulation and N fixation by rhizobium on legumes Johansen *et al*. (1992). The existence of mycorrhizal for the availability of nitrogen, phosphorous and potassium on entisol absolutely necessary (Abdollahi *et al*., 2015; Buntan *et al*., 1997; Mosse, 1981).

Physically the soil has a large stable aggregate, either macro or micro. The AMF external hyphae which develop into the soil can bind soil particles and form aggregates, so that the amount of soil particles are degraded far more than mycorrhizal plants. The formation of stable soil aggregates with AMF is an important factor in increasing the fertility of soil physically (Baon, 1998).

Alternative technologies that can be used to increase the production of food crops, horticulture, plantation, as well as medicinal plants is through microbes associated with the plant. Mycorrhiza as bio-fertilizer has the ability to improve plant growth and development. Besides, it also can act as bio-protector against the occurrence of pests and diseases. One of mycorrhizal is AMF through spores that infect plants, grows and develops in the cortex. This fungus morphology consists of arbuscel, vesicles, internal and external mycelium. Advantage of AMF is its ability to help the plants to absorb nutrients especially P nutrient (Syafruddin *et al*., 2010; Syib'li, 2008a, b).

Musfal (2010) stated that the FMA application on corn in the Inceptisols can improve root infection, phosphate uptake, plant dry weight and dry seed. Additionally, FMA can increase the absorption of macro and micro nutrients in the presence of external hyphae and improve nutrient availability as a result of the activity of enzymes that help to release fixed nutrients. In addition, it has been widely reported that the FMA is able to increase the resistance of plants to drought (Zhu *et al*., 2012; Setiadi, 1999; Johnson and Wedin, 1997). Therefore, corn is usually to use as a host plant that could stimulate root and shoot biomass (Table 3 and 4).

The AMF has considerable potential in increasing the sustainability of agricultural ecosystems through its role in improving plant nutrient cycle and the improvement of soil aggregates. This process can result in the ability to grow better seeds, plant biodiversity and productivity (Sieverding, 1991). According to Abbott and Robson (1984), mycorrhizal infectivity is as power or ability of mycorrhizal fungi to infect and colonize plant roots. In that sense encompassed the development and activity of the fungus before and after infection occurs, meaning intended infectivity can also be referred to as a measure of a succession of fungi interact with the plant and soil conditions. Infectivity in this case is expressed as a proportion of the roots of infected plants (Giovannetti and Mosse, 1980).

Improvement of crop yield with application of AMF is also associated with mycorrhizal repair itself. Although in some cases this fungus increase crop yields but failed to improve the status of mycorrhizal. At the same time, in some cases AMF failed to improve crop yields, although they are able to improve the condition of mycorrhizal (Kehri and Chandra, 1990).

Environmental and biotic factors have an influence on the formation of AMF and the degree of infection of the host cell cortex. Differences in the time required for the infection is caused by several factors, among others: Root density, average root growth, the No. of spores/unit volume of soil, spore germination percentage and the average growth of the hyphae. Interactions between biotic factors have a significant effect in response to the growth of the inoculated plants. Environmental factors influence the formation of AMF in terms of supply and balance of nutrients, moisture and soil pH (Rao, 1994).

The AMF infection due to an increase in the percentage of inoculation can be attributed to an increase in the No. of spores in the soil. Infection occurs because of an exudate or characteristic compounds produced and secreted by plant roots which led to the development of AMF increased. An increase in the percentage of infection due to inoculation is strongly influenced by the rhizobium (Fakuara, 1998). Differences in location and rhizosphere causes species diversity and population differences of AMF. Land dominated by clay fraction (clay) is a condition that is believed to correspond to the development of Glomus spores and in sandy soil Gigaspora genus are found in high amounts. In sandy soil, the soil has more macro pores than the clay and is believed to correspond to the state of development of the spores Gigaspora which is larger than the spores of Glomus (Baon, 1998).

Succession AMF generally affected by plant age, the highest infection primarily in the roots of young plants and other factors that influence environmental factors such as light, soil fertility (element P and N available), water content, soil drainage and soil pH. In addition, AMF can thrive in soil that has a lower P content and better soil aeration (Husein *et al*., 2000). Barrett *et al*. (2011) also concluded that the arbuscular mycorrhizal fungus Glomus can capture and transfer nitrogen from organic patches to its associated host plant at low temperature. Deployment of AMF can occur through a variety of ways, active deployment mycelia through the soil. Through the deployment of mycorrhizal inoculation slightly reduced in mycorrhizal soil already, but increased in non-mycorrhizal soil. In o ther words, the new AMF will be spread on soils low mycorrhizal content. While passive deployment can be done by some animals and also by wind (Suhardi, 1989; Setiadi, 2001).

Whether there is a suitable host plants turned out to affect the presence or absence of colonies AMF and

production of spores, arbuscular mycorrhizal fungi are not really a type of fungus that is very specific to a particular host plant. Perhaps one species can be more efficiently formed an association on certain plants, but actually AMF fungus can form colonies on almost every host plant. The further study is possible that there is only a AMF species germinated on the type of host plant only limited and specific environmental conditions, but this is only an exception. The AMF is known to associate with a variety of plants angiosperms, both dicots and monocots, annual or seasonal crops and plants locally (Fitter and Hay, 1991; Lakitan, 2000).

CONCLUSION

Based on the potential degree of root infection, spore populations and root biomass produced we concluded that propagation techniques on mycorrhizal bio-fertilizer in entisol should be used with starter types of mycorrhizal *Glomus mosseae*+*Gigaspora* sp. and the host plant of corn. Other alternatives can also be done using a starter *Gigaspora sp*. and the host plants of white sorghum. Subsequent study on application the produced mycorrhizal bio-fertilizer to increase the production of chili at entisol is absolutely necessary.

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