Jurnal Natural
Vol. 18 (2) 2018
DOI 10.24815/jn.v18i2.11061
Published June 2018
pISSN 1411-8513
eISSN 2541-4062

THE EFFECT OF ARBUSCULAR MICHORRIZA FUNGI AND ORGANIC FERTILIZER ON THE GROWTH OF SUNFLOWER (HELIANTHUS ANNUUS L.) ON CRITICAL LAND OF FORMER IRON ORE MINE IN LHOONG - ACEH BESAR

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Abstract. The study determines the effect of arbuscular mycorrhiza fungi (AMF) and organic fertilizer on sunflower growth (Helianthus annuus L.) in former iron ore mine in Lhoong, Aceh Besar. The Randomized block design is 2×3 factorial with 4 replications was used. The first factor: Without Mycorrhiza and Mixed Mycorrhiza. The second factors were Without Fertilizer, Manure, and Guano. The results showed that at α = 5% variation analysis, AMF had very significant effect on plant height in 10 d After Planting (DAP), leaf number 50 DAP, fresh weight of top-trimmed, and AMF colonized roots. Furthermore, it showed a significant effect on plant height 40 and 50 DAP, leaf number 20, 40 DAP and dry weight of top-trimmed. Fertilizer were significantly affected on plant height in 10 and 20 DAP, leaf number 50 DAP and fresh/dry weight of the top-trimmed. It showed a significant effect on plant height 30 DAP and leaf number 40 DAP. Mixed Mycorrhiza and Guano showed the best results. The interaction between AMF and fertilizer had a significant effect on plant height at 50 DAP and fresh weight of the top-trimmed. Mixed Mycorrhiza and Manure showed the best results on 50 DAP with increasing in plant height, while Mixed mycorrhiza and Guano increased fresh weight of the top-trimmed.

Keywords: arbuscular mycorrhiza fungi (AMF), Helianthus annuus L., critical land, organic fertilizer, sunflower growth.

I INTRODUCTION

Land degradation is a process where the value of the biophysical environment is influenced by one or more combinations of human action processes to the soil [1]. In addition to natural disasters, an indirect human activity can be also affected the natural phenomena such as floods and forest fires that can lead to land degradation. It is estimated that up to 40% of agricultural land in the world is seriously degraded into the critical land. In Aceh, there are many mine sites that leads to many critical lands. One of the places that seriously affected the agricultural and economic output of the community is iron ore mine in Lhoong Sub-district, Aceh Besar District. Approximately 500 ha of territory within the two settlements, namely Mukim Cot Jeumpa and Mukim Blang Me, had been exploited into an iron ore mine land. The exploitation area is located in four villages, namely Gampông Jantang, Gampông Baroh Blang Me, Gampông Baroh Geunteut, and Gampông Teungoh Geunteut. [2]. The impact felt by the community. For instance the well and the river becomes dry, the plants were difficult to grow, the rice fields become damaged which all have a negative impact on the economic income of society and ecology. So there must be an effort to tackle the critical land and find a quick and precise solution. One of solution is by adding organic materials to improve the physical, chemical and biological properties of the soil. Organic materials also contribute a lot of nutrients such as N, P, K, Ca, Mg and increase the availability of other nutrients for plants. The presence of organic matter also increases the population and activity of organisms in the soil

that can produce humic acid, comic acid, carboxyl, phenol and other organic acids. These compounds can react with Al and Fe, so that more plant nutrients are available [3]. The role of mycorrhiza as a biological fertilizer in increasing the availability of nutrients in the soil is also a challenge. The mycorrhiza genus widely used as a biological fertilizer is *Glomus* sp., *Acaulaspora* sp., *Gigaspora* sp. and *Scultellospora* sp. obtained from host plants [4]. Critical soil can be utilized by planting crops that have high adaptation to low soil fertility conditions. One such plant is a sunflower. Sunflower plants can be found in disturbed habitats, newly opened environments and various meadows. Sunflower plants grow all year round in all places around the world [5]. Sunflower plants are one source of biodiversity that acts as phytoremediation and biofuel producer. Phytoremediation using crops and associated microbes is increasingly being used as a green technology to clean contaminated soil of mine. Provision of AMF in host plants (sunflower plants) is able to reduce the toxicity of contaminated mining land. Inoculation of AMF (R. irregularis) can be for Cd pho-extraction while F. mosseae may be useful for phytostabilization of Cd and Zn [6]. Based on the study of the potential of AMF, organic fertilizer and sunflower benefits are quite promising, the authors tried to use mycorrhiza and organic fertilizer to see how the effect of AMF and organic fertilizer on the growth of sunflower plants on critical land of former iron ore mine and this research is expected to help increasing growth of sunflower on such land in Lhoong Aceh Besar.

II METHODOLOGY

This research was conducted in Meunasah Papeun Village, Krueng Barona Jaya Sub-district, Aceh Besar District. Soil and cultivated soil as well as guano were analyzed at Soil and Plant Laboratory, Faculty of Agriculture, Syiah Kuala University. Moreover, the percentage test of plant root colonization by mycorrhiza was done in Soil Biology Laboratory of Soil Department, Faculty of Agriculture, Syiah Kuala University. The research took place from January to December 2017. Furthermore, the soil material used was critical soil taken from the former iron ore mine in Lhoong of Aceh Besar Regency. Then, Inoculum AMF used was Mixed Eclipse (*Glomus*, *Gigaspora*, and *Acaulospora*) with density of 100 spores 10 g^{-1} obtained from Soil Biology Laboratory of Soil Department, Syiah Kuala University. While, Sunflower seedlings of local varieties are obtained from Gampong Lhong Raya, Banda Raya Sub-district, Banda Aceh. Organic fertilizer consists of manure obtained from the Business Area Stores at Diponegoro Street, Banda Aceh. Last, guano fertilizer was taken from the bat cave around Lamdom village, District Lampuuk, Aceh Besar. The basic fertilizer (manure) consists of KCl, TSP, and Urea. Materials for analysis in the laboratory, namely: 5% vinegar, Quink blue, sterile aquades, 70% alcohol, KOH, Bayclin, and others. Some equipment used were measuring glass, object glass, glass cover, glass cup, stir bar, light microscope, cutting knife, analytical scale, meter and polybag measuring 40 × 50 cm, hoe, bucket, and others. This research is a pot experiment in the field organized according to Randomized Block Design (RBD) 2 × 3 factorial pattern with 4 replications. There are two factors studied, the first factor is the use of AMF as much as 10 g $plant^{-1}$ consisting of 2 types, namely: M0 = Without Mycorrhiza and M1 = Mixed Mycorrhiza. The second factor is organic fertilizer with a dose of 30 ton ha^{-1} or equivalent to 150 g of $polybag^{-1}$, consisting of 3 types, namely: P0 = Non-fertilizer, P1 = Manure, P2 = Guano. From these two factors, there were 6 treatment combinations and each treatment combination was repeated 4 times to 24 units of the experiment. Planting was done in a polybag containing 10 kg of soil. The mathematical model of Randomized Factorial Randomized Design is as follows:

$$Y_{ijk} = \mu + \beta_i + M_j + P_k + (MP)_{ijk} + \acute{\varepsilon}_{ijk} \quad (1)$$

Data analysis to know the diversity of each observation parameter was done by using variance analysis (F test) at $\alpha = 5\%$ by using *Statgraphics for Windows* software. If there is a significant difference the analysis is continued with Least Significant Difference (LSD) at 5%.

Soil Analysis

The soil analysis was conducted to find out the characteristics of some soil properties studied before the experiment. The properties analyzed were: pH H_2O, C-organic (Walkley&Black method), N-total (Kjeldhal method), P-available (Bray II method), K-dd, Ca-dd, Mg-dd, Na (SDN extraction method (NH_4OAc pH7 extraction method), EC (Electricity Conductivity).

Analysis of Organic Fertilizer

Fertilizer analysis included the C-organic content (Walkley&Black method), N-total (Kjeldhal

method), P-total and K-total (25% HCl destruction method). The determination of P and K in the extract solution was carried out each with a spectrophotometer and flame photometer.

Land preparation
Soil material was taken at a depth of 0-20 cm, cleaned, dried, mashed and sieved used a 2 mm diameter of hole size sieve. Furthermore, the soil is filled into the polybag each of 10 kg and watered sufficiently.

Providing organic fertilizer treatment
Provision of organic fertilizers performed according to treatment a week before planting (incubation period). The way of giving it was by mixing the average organic fertilizer with soil in each polybag as much as 150 g.

Planting
Firtsly, the contaminated soil was fullfilled into all 10 g polibags. Planting holes were made as deep as 5 cm on the top surface of the soil. Thus, the mycorrhiza was put into the hole. Furthermore, 3 sunflowers were planted in the polybag. After the age of one week, the plants were selected to live one of the best growth, while the other wereis cut and buried into the soil.

Fertilization
The basic fertilizer in each soil polybag was given with KCl of 50 kg ha^{-1} or 0.25 g $polybag^{-1}$, TSP 100 kg ha^{-1} or 0.5 g $polybag^{-1}$ and Urea 50 kg ha^{-1} or 0.25 g $polybags^{-1}$.

Observation
Parameters observed: (1) plant height, (2) number of leaves. Both parameters are measured from 10 Days After Planting (DAP) with the time interval for next 10 DAP until 50 DAP. Other parameters tested were (3) fresh weight of top-trimmed, (4) dry weight of top-trimmed, (5) percentage of AMF colony root by Phillip and Hayman Method (1970) modified in Nusantara (2007) [7]. For the 3^{rd} to the 5^{th} parameter is done at plant age 55 DAP.

III RESULT AND DISCUSSION

Plant height
The result of variance analysis (F test) showed that AMF was very significant at 10 DAP, not significant at 20 and 30 DAP, and had a significant effect on 40 and 50 DAP on plant height. While the provision of organic fertilizer had a very significant effect on 10 and 20 DAP, the effect was significant on 30 DAP and had no significant effect on 40 and 50 on DAP of plant height. Giving AMF with organic fertilizer showed a significantly different interaction at 50 DAP of plant height. The average value of plant height due to the treatment of AMF and organic fertilizer can be seen in Table 1. Symbiotic AMF plays an important role in the early colonization of soil by plants [8]. Table 1 shows that at 10 DAP, AMF has a very significant effect and also a significant effect shows on 40 and 50 DAP of plant height. Plants given Mixed Mycorrhiza are higher than those without Mycorrhiza, i.e. 12.50 cm compare to 10.32 cm at 10 DAP, 39.38 cm compare to 36.86 cm at 40 DAP (Figure 1), and 53.96 cm compare to 49.71 cm at 50 DAP. The role of AMF helps plants to capture nutrients such as phosphorus and soil micronutrients. According to Ref. [9], phosphorus plays a role in cell division, root development, flower and seed formation, RNA and DNA compounds and energy storage (ATP and ADP). Kalium works to increase the activity of enzymes, protein synthesis, absorption, ion transport and in photosynthesis as well as in process of respiration.

Figure 1 The difference of plants height at 40 DAP.

Table 1 shows that at 10 and 20 DAP the effect of organic fertilizers was very significant different and had a significant effect on 30 DAP of plant height. Provision of organic fertilizer as a single factor at 10 DAP showed the best result was Without Fertilizer (12.38 cm), eventhough as statistically showed no significantly different with the provision of Manure (12.06). While Guano showed the lowest yield on plant height (9.79 cm). Provision of organic fertilizer on a single factor at 20 and 30 DAP showed the best result was Manure (21.63 cm and 30.63 cm respectively), although statistically not

significantly different with Non-Fertilizer (20.38 and 29.50), while Guano showed the lowest yield on plant height (16.52 cm and 25.85 cm). Brady (1990) explains that the decomposition of organic fertilizer, when incorporated into the soil, will produce some nutrients needed by plants such as N, P, and K. The good nutrient absorption by this plant is also strongly influenced by AMF which begins to show a real interaction with organic fertilizer at 50 DAP as shown in Figure 2. The figure shows that at 50 DAP mycorrhiza produces the highest crops when interacting with Manure i.e. 57.13 cm, but produces low crops when interacting with Guano i.e. 49.75 cm. The most prominent AMF function is its ability to associate with almost 90% of the plant species. Nutrient content in manure is more than in guano, especially nitrogen that can increase plant height. According to Ram (2007), the benefits of nitrogen are to stimulate the growth of plants in the vegetative phase and play a role in the formation of chlorophyll, amino acids, fats, enzymes and other compounds. De La Cruz (1981) proves that CMA is capable to replace approximately 40% nitrogen.

Leaf Number

The results of variance analysis (F test) showed that AMF provision had no significant effect on 10 and 30 DAP, but significant affecting at 20 and 40 DAP, and highly significant effect at 50 DAP on the number of plant leaves. While the provision of organic fertilizer had no significant effect at 10, 20, and 30 DAP, significant effect at 40 DAP, and very significant effect at 50 DAP. Giving AMF with organic fertilizer didn't show a real interaction. The average value of the number of leaves of sunflower plants due to the treatment of AMF and organic fertilizer can be seen in Table 2

Table 1 The average plant height after AMF and organic fertilizer treatment

Measurement Time	Treatment	Plant Height (cm)			Mean
		Without Fertilizer	Manure	Guano	
10 DAP	Without Mycorrhiza	10.88	11.25	8.83	10.32 A
	Mixed Mycorrhiza	13.88	12.88	10.75	12.50 B
	Mean	***12.38 b***	***12.06 b***	***9.79 a***	
20 DAP	Without Mycorrhiza	18.88	20.25	15.67	18.26 A
	Mixed Mycorrhiza	21.88	23.00	17.38	20.75 A
	Mean	***20.38 b***	***21.63 b***	***16.52 a***	
30 DAP	Without Mycorrhiza	28.50	29.38	25.33	27.74 A
	Mixed Mycorrhiza	30.50	31.88	26.38	29.58 A
	Mean	***29.50 b***	***30.63 b***	***25.85 a***	
40 DAP	Without Mycorrhiza	36.13	37.13	37.33	36.86 A
	Mixed Mycorrhiza	39.25	42.38	36.50	39.38 B
	Mean	***37.69 a***	***39.75 a***	***36.92 a***	
50 DAP	Without Mycorrhiza	46.38	49.75	53.00	49.71 A
	Mixed Mycorrhiza	55.00	57.13	49.75	53.96 B
	Mean	***50.69 a***	***53.44 a***	***51.38 a***	

At 10 DAP: LSD mycorrhiza = 0.850, LSD of fertilizer = 1.042
At 20 DAP: LSD of fertilizer = 3,103
At 30 DAP: LSD fertilizer = 2,664
At 40 DAP: LSD mycorrhiza = 2,412
At 50 DAP: mycorrhizal LSD = 3,263
Description: The numbers followed by the same letter in the same column and row are not significantly different at the P level ≤ 5%. The lower case is read horizontally and the uppercase is read vertically.

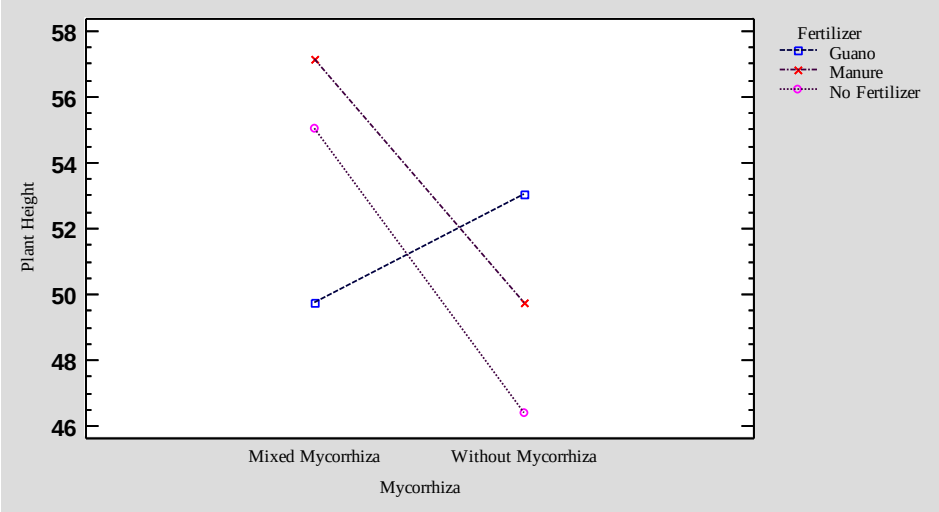

Figure 2 Interaction AMF and Organic Fertilizers on plant height at 50 DAP

Table 2 The average number of sunflower plant leaves after of AMF and organic fertilizer treatment

Measurement Time	**Treatment**	**Number of leaves**			**Mean**
		Without Fertilizer	**Manure**	**Guano**	
10 DAP	Without Mycorrhiza	7.00	6.50	6.67	6.72 A
	Mixed Mycorrhiza	7.50	8.00	7.00	7.50 A
	Mean	***7.25 a***	***7.25 a***	***6.83 a***	
20 DAP	Without Mycorrhiza	9.75	10.50	10.67	10.31 A
	Mixed Mycorrhiza	11.25	11.25	10.50	11.00 B
	Mean	***10.50 a***	***10.88 a***	***10.58 a***	
30 DAP	Without Mycorrhiza	13.75	15.25	16.67	15.22 A
	Mixed Mycorrhiza	15.25	17.50	17.00	16.58 A
	Mean	***14.50 a***	***16.38 a***	***16.83 a***	
40 DAP	Without Mycorrhiza	18.25	22.00	22.33	20.86 A
	Mixed Mycorrhiza	20.75	24.50	23.25	22.83 B
	Mean	***19.50 a***	***23.25 b***	***22.79 b***	
50 DAP	Without Mycorrhiza	23.00	29.75	28.67	27.14 A
	Mixed Mycorrhiza	25.75	31.50	31.25	29.50 B
	Mean	***24.38 a***	***30.63 b***	***29.96 b***	

At 20 DAP: mycorrhiza LSD = 0.655,
At 40 DAP: LSD mycorrhiza = 1.809, LSD of fertilizer = 2,216
At 50 DAP: LSD mycorrhiza = 1,788, LSD of fertilizer = 2,189
Description: The numbers followed by the same letter in the same column and row are not significantly different at the P level ≤ 5%. The lower case is read horizontally and the uppercase is read vertically.

Table 2 shows that mixed mycorrhiza had a significant effect at 20 and 40 DAP, and very significant effects at 50 DAP in increasing the number of leaves. The provision of organic fertilizer was significantly different at 40 DAP. The best result was Manure, although it was not statistically different with Guano, while Non-Fertilizer showed the lowest result on increasing the number of plant leaves. The provision of organic fertilizer has a significant different effect at 50 DAP and the best result was manure although it was not statistically different with guano whereas without fertilizer showed the lowest result in increasing the number of plant leaves. This is possible since the nutrient content of manure is more varied and more readily N available for the growth of crops for high growth and Mn for leaf shoot growth. Manure contains macro and micronutrients such as phosphorus, nitrogen, potassium, calcium, magnesium, sulfur, sodium, iron, copper, and molybdenum [10]. Guano contains higher P elements compared to N and K nutrients, while in vegetative phase growth the plants require more nitrogen than phosphorus according to Ref. [11] which states that the most nutrient factor affecting leaf growth and development is N.

Fresh /Dry Weight of Top-Trimmed

The result of variance analysis (F test) showed that the giving of AMF and organic fertilizer respectively had a very significant effect on the fresh weight of the crops and showed the interaction of the two factors that had a real effect. The treatment of AMF had a significant effect on the dry weight of crops and organic fertilizers, but it did not show the interaction of the two factors. The average value of fresh weight and dry weight of the top tack can be seen in Table 3. The table shows that fresh weight and dry weight of top-trimmed that were given as a single factor Mixed AMF treatments, each showing the best response (28.65 g and 4.49 g) compared to Without Mycorrhiza (24.17 and 3.81 g). While the provision of organic fertilizer as a single factor, Guano, that gives the best results on the increase of fresh weight of the top-trimmed (39.92 g) and dry weight top- trimmed plants (5.98 g). This is very possible because the Guano fertilizer contains many elements of P that play a role in cell division [9]. The interaction of the two factors to the fresh weight of the top-trimmed is shown in Figure 2.

Table 3 The average fresh weight and dry weight of the plants after of AMF and organic fertilizer treatment

Measurement Parameter	Treatment	The weight of the top trim (g)			Mean
		Without Fertilizer	Manure	Guano	
Fresh weight	Without Mycorrhiza	5.94	26.20	40.37	24.17 A
	Mixed Mycorrhiza	15.07	31.42	39.47	28.65 B
	Mean	***10.51 a***	***28.81 b***	***39.92 c***	
Dry weight	Without Mycorrhiza	1.53	3.88	6.03	3.81 A
	Mixed Mycorrhiza	2.85	4.68	5.93	4.49 B
	Mean	***2.19 a***	***4.28 b***	***5.98 c***	

On Wet Weight: LSDMycorrhiza = 2.558, LSDpupuk = 3,133
On Dry Weight: LSDMycorrhiza = 0.742, LSDpupuk = 0.578
Description: The numbers followed by the same letter in the same column and row are not significantly different at the P level ≤ 5%. The lower case is read horizontally and the uppercase is read vertically.

Table 4 The mean percentage of AMF colonized root after AMF and organic fertilizer treatment

AMF	Colonized root (g)			Mean
	Without Fertilizer	Manure	Guano	
Without Mycorrhiza	0.50	2.50	3.33	2.11 A
Mixed Mycorrhiza	8.00	6.75	6.75	7.17 B
Mean	**4.25**	**4.63 a**	**5.04 a**	

LSD mycorrhiza = 2,710
Description: The numbers followed by the same letter in the same column and row are not significantly different at the P level ≤ 5%. The lower case is read horizontally and the uppercase is read vertically.

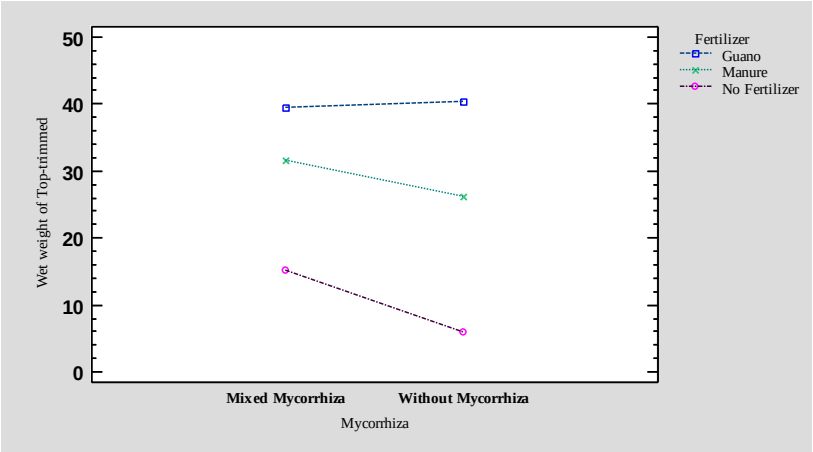

Figure 3 Mixed Interaction among Organic Fertilizer on fresh weight of the top-trimmed.

Figure 3 show that the combination of Mixed mycorrhiza with Guano gives the best response to the increase of fresh weight of the top-trimmed (39.47 g), then combination of the Mixed Mycorrhiza and the Manure (31.42 g). The lowest number is the Mycorrhiza Mixed and Without Fertilizer (15.07 g). This is the evidence of both AMF and organic fertilizer has played an important role in improving plant growth. In addition, the provision of organic fertilizer will produce humic and fulvic acid which plays an essential role in the bonding of Fe and Al which is soluble in the soil, so that the availability of P will increase. AMF also plays an important role in maintaining the stability of plant diversity by transferring nutrients from one plant to another adjacent plant through a structure called "bridge hypha" [12]. De La Cruz (1981) in Octavitani (2009) proves that AMF is capable of replacing approximately 50% of phosphate, 40% nitrogen, and 25% potassium.

Percentage of AMF Colonized Root.

The result of variance analysis (F test) showed that AMF significantly influenced the percentage of AMF colonized root, while organic fertilizer was not significantly different and there was no interaction between the two factors. The average value of AMF colonization root percentage due to the treatment of AMF and organic fertilizer is shown in Table 4. The table shows that Mixed Mycorrhiza (7.17 g) gives more colonized root than Without Mycorrhiza (2.11 g). This indicates that AMF can also help plant growth in heavy metal contaminated soils such as post-mining contaminated land [13]. Mycorrhizal rather than non-mycorrhizal plants could colonize polluted mining sites [14]. For that AMF can be used as bio-protection, it also serves as an important bio-remediator for heavy metal polluted soils [13]. Table 4 also shows that soils naturally contain mycorrhiza. This is consistent with Linderman's (1988) assertion that there is an infection in plants that are not given mycorrhiza because in the planting medium used there is still active indigenous mycorrhiza propagules. Germination of inoculated fungal amorphous fungi stimulated by the presence of root exudates. It is confirmed by Mansur (2003) that mycorrhiza spores can last up to two years before germination. The population of these fungi in the soil media will affect the infection in the roots or increase the colonization of fungi within the roots of the plant.

CONCLUSION

Provision of AMF and organic fertilizer have significantly affect to the growth of sunflower plants on the critical land of the former iron ore mine in Lhoong - Aceh Besar. The interaction of these two factors proved in several growth parameters. On plant growth showed that AMF has a very significant effect on plant height at 10 DAP, leaf number at 50 DAP, fresh weight of top-trimmed, AMF colonized root and significant effect on plant height at 40 and 50 DAP, leaf number 20 and 40 DAP and dry weight top-trimmed. The provision of organic fertilizer had a very significant effect on plant height at 10 and 20 DAP, leaf number at 50 DAP, fresh weight and dry weight of top-trimmed, significant effect on plant height at 30 DAP and leaf number 40 DAP. The interaction of both factors had a significant effect on plant height at 50 DAP and fresh weight of the top-trimmed. Giving a mixed mycorrhiza, guano fertilizer and their combination showed the best results on the growth of sunflower plants on the critical land of the former iron ore mine in Lhoong, Aceh Besar.

ACKNOWLEDGMENT

Our immense gratitude to the Jantang Village Leader and villagers who have allowed and assisted us in researching ex-mine of iron ore fields in their village. They are eager to know how to heal the soil that can no longer be planted. Then the infinite gratitude to Mrs. Fikrinda, from the Agricultural Faculty of Syiah Kuala University, which tirelessly guided us until the research was done perfectly. Our appreciation is also to Soil Laboratory of Agriculture Faculty officers and Soil Laboratory officers in BPTP Aceh who has helped to analyze the soil properties before and after the growth of sunflower plants. Our highly appreciation also tribute to Ristekdikti as funding of the research. Best thanks to my family (my husband and my lovely kids: Arib, Diva, Hafidh and Ryan).

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