3rd Malikussaleh International Conference on Multidiciplinary Studies 2022 (3rd MICoMS 2022)

IINTERNATIONAL CONFERENCE PROCEEDINGS 00058 (2022) | DOI: https://doi.org/10.29103/micoms.v3i.222
E-ISSN: 2963-2536

The Important Roles of Ecomychorizae to Increase Growth Rate of Sacha Inchi (*Plukenetia volubilis* L.) that Potentially as Raw Material of Biofuel

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ABSTRACT
Recently the use of agricultural waste containing cellulose and lignin as an alternative fuel that is environmentally friendly. The study aimed to determine the important roles of eco-enzymes and mycorrhizae due to increasing the growth rate of sacha inchi plants that are tolerant to nutrient-poor soils such as Inceptisol which are classified as sub-optimal lands. Sacha inchi broadleaf plants are classified as having a high lignin content due to their biomass potentially producing alternative fuels that can reduce dependence on the use of fossils, which were decreasing. This research was conducted in West Reuleut Village, Muara Batu District, North Aceh District and the Laboratory of the Faculty of Agriculture, Malikussaleh University. This research was carried out from April to August 2022.The Application of eco-enzymes on Inceptisol soil has increased the absorption of several macronutrients that were impacted by the fresh weight of the root, root dry weight, and root length of sacha inchi. The activities of eco enzyme and mycorrhizae which were giving a positive impact on the vegetative growth rate of Sacha inchi plants. Eco enzymes contain microorganisms that help the process of decomposition, transport of nutrients, and mycorrhizae as biological fertilizers that can provide nutrient uptake for plants. The impact of synergizing both fertilizers together has increased the suitability and productivity of sub-optimal soil that is able to increase growth and yield plant. The increasing productivity of Sacha inchi plants which contain lignin and cellulose also increases plant biomass which has the potential to produce high bioethanol as well.

Keywords: *sub-optimal land, classification, productivity*

1. INTRODUCTION

Sacha inchi (*Plukenetia volubilis* L) is a broad-leaf plant that contains high fiber and lignin. Sacha inchi originating from Peru [1]Hidalgo *et al*., 2019, is nowadays widely cultivated in Asia. This plant has a broad leaf belonging to the Euphorbiaceae family [2]Nisa *et al*., 2017. Plunetia volubilis is a woody vine that produces seeds with high protein content (27–30%) and oil (40–60%), exceeding the quality characteristics of the oil consumed around the world [3]Cai *et al*., 2013. Plant specialty Sacha inchi is the content of unsaturated fatty acids that beneficial as nutritional food which is rich in omega-3 by 48.61%, higher compared to other plants. [4]Hamaker *et al*. (1992), report that sacha inchi has many nutrients very beneficial for the body because it contains omega 3 45.2%, omega 6 36.8%, omega 9 9.6%, and 7.7% saturated fat. Sacha inchi waste potentially produces biofuels. The biomass of this plant especially the shell contains high lignin. More detailed features of Sacha inchi, can be shown in Figure 1.

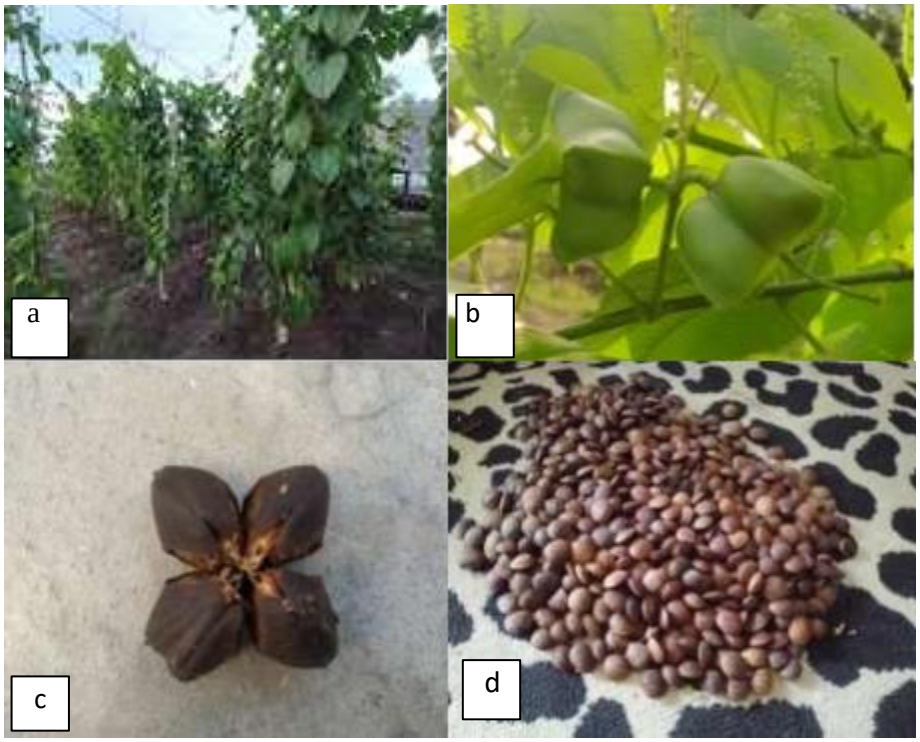

Figure 1. (a) Sacha inchi young fruit, (b) Mature fruit of sacha inchi, and (d) Sacha inchi seeds

Sacha inchi tolerant to cultivate in sub-optimal land such as Inseptisol soil Reuleut due to low productivity due to environmental stress (low fertility, limited groundwater, lack of organic matter and acidity) there for categorized as marginal lands. Furthermore [5]Siswanto & Widowati (2018), mentioned the low fertility of inceptisols is of water stress, lack of soil organic matter content, and low pH.

To increase the availability of several macro elements in the soil that can be improved Inceptisol soil can be done by adding organic matter and biological fertilizers such as the application of eco -enzymes and Arbuscular Mycorrhizal Fungi (AMF). Eco-enzyme contains the macro elements potassium (K) at 203 mg/l and phosphorus (P) at 21.79 mg/l [6]Yuliandewi *et al*., 2018 so that it can improve the characteristics of inseptisol soil. [7]Jaya *et al*. (2021), in their research a concentration of 22.5 ml/l of eco-enzyme had a significant effect on the fresh weight of tubers per clump of shallot plants. The content of eco enzymes and mikorrizae as biofertilizers also facilitates plant growth, stimulates fruiting, and improves the quality of f ruits and vegetables.

Mycorrhizal is a form of mutualism symbiosis between AMF hyphae and plant root systems so that it can help the availability and absorption of plant nutrients, and plant yields. According to [8]Sufardi (2012), the mycorrhizal colonization effect expands the root zone up to 80 times, thus increasing the absorption rate of nutrients to four times compare to normal roots. [9]Marwani *et al.* (2013), stated that the application of mycorrhizal 30 g increased the absorption of elements N, P, K, Mg, and Ca give significan tly affected plant height. The use of eco-enzymes and mycorrhizae gave a positive response to the improvement of inceptisol soil characteristics which can be seen in the increase in the vegetative growth rate of sacha inchi.

2. RESEARCH METHODS

This research was conducted in West Reuleut, Muara Batu District, North Aceh District, and the Laboratory of the Faculty of Agriculture, Malikussaleh University. This research was Carried out from April to August 2022.

2.1. Materials and Equipment

The materials used for this research were sacha inchi plant seeds, topsoil, water, shallots, cow manure, NPK fertilizer, biochar, Sawdust, sand, eco-enzyme, micophere (the material containing mycorrhizal spores) with 99 spores/100 g micopheres containing spores (*Glomus claroideum, Acaulospora rogusa, Acaulospora colosica, Glomus fasciculatum, Glomus mosseae, dan Glomus etunicatum*), distilled water, potassium hydroxide 10% hydrochloric

acid, and methylene blue 0.02%. The tools used in this study were agricultural tools, paranet 80%, scissors, oven, digital scales, plastic bags, 2 m markers, and stationery.

2.2. Experimental Design

The study used a factorial randomized block design with two treatment factors, namely eco-enzyme (E) concentration of eco-enzyme (E) consisting of 3 levels: E0 (0 ml/l), E1 (22.5 ml/l), E2 (30ml/l) F and micofer (M) namely: M0 (0 g/plant), M1(30 g/plant), M2 (40 g/plant). Thus there are 9 treatment combinations with 3 repetitions so there are 9 x 3 = 27 experimental units. Then in each bed there were 6 research plants, so that a total of 27 x 6= 162 plants was obtained.

The implementation of this research consisted of making eco-enzymes, sowing seeds, preparing land, cultivating soil, making beds, installing stakes, planting, applying mikofers, applying fertilizers eco enzymes, maintenance. While the variables observed in this study were Root Length (cm), Root Fresh Weight (g), Root Dry Weight (g) and Mycorrhizal Infection (%). Sacha inchi plant maintenance includes watering, weeding, pest control, replanting, and fertilizing. Watering of plant done in the afternoon. Weeding done by clearing the weeds that grows surrounding the plants to avoid competition between the weeds and the sacha inchi plants.

3. RESULTS AND DISCUSSION

3.1. Plant Length (cm)

The concentration eco-enzyme alone showed a very significant effect at the age of 10 and 20 days after the planting (DAP). The concentration of E1 produced the best plant length, which was 66.14 cm, which was significantly different from E0 (control). A single dose of mycorrhizae showed a very significant effect of age on plant length variables, namely at the age of 10, 20, 30, 40, 50, and 60 days after planting. The dose of 40 g as the best plant length at 284.44 cm, which was significantly different without mycorrhizae fungi application.

Table 1. Root length as a result of *eco-enzyme* concentration and Mycorrhyzae doses.

Treatments	Plant length (cm)					
	10 HST	20 HST	30 HST	40 HST	50 HST	60 HST
Eco-enzyme (E)						
E0 (0 ml/l)	45.48 b	49.31 b	70.04 a	113.80 a	177.74 a	224,15 a
E1 (22,5 ml/l)	61,64 a	66.14 a	80.57 a	128.43 a	207.54 a	274.00 a
E2 (30 ml/l)	57.90 a	63.25a	85.30 a	127.27 a	197.50 a	263.63 a
Mycorrhizal (M)						
M0 (0 g/plants)	43.40 b	47.00 b	59.65 b	9165 b	151.28 b	203.67 b
M1 (30 g/plants)	56.59 a	61.17 a	80.07 a	132.33 a	197.56 a	268.67 a
M2 (40 g/plants)	65.04 a	70.53 a	96.18 a	145.51 a	233.94 a	284.44 a

Note: The numbers followed by the same letter in the same column are not significantly different according to the DMRT 5 test

3.2. *Root Length (cm)*

There was a significant interaction between the concentration of eco-enzyme and dose of mikofer on root length variables. The best root length was obtained in the E1M2 treatment interaction with an average root length of 58.39 cm, while the lowest number of leaves was in the E0M0 treatment interaction with an average root length of 31.64 cm (Table 4).

The concentration *eco-enzyme* alone did not show a significant effect on root length variables. E2 concentration produced the best root length, which was 51.55 cm that was significantly different from E0 (control) with an average root length of 26.25 cm. Micofer dose alone showed a significant effect on root length variables. The M2 dose produced the best root length, which was 55.30 cm, which was significantly different from M0 (control) with a root

length of 44.16 cm. The Figure of root length due to mycorrhizal colonization and in the absence of mycorrhizal colonization as depicted in Figure 2.

Figure 2. (a) roots without mycorrhizal colonization, (b) roots with mycorrhizal colonization 40 g/plants mycorrhizal

3.3 Fresh Weight of Roots (g)

There was a significant interaction between the concentration of eco-enzyme and the dose of micoferre on the root fresh weight variable. The best root fresh weight was obtained in the E0M2 treatment interaction with an average root fresh weight of 44.52 g, while the lowest fresh weight was in the E0M0 treatment interaction with an average root fresh weight of 7.47 g (Table 6).

The concentration *of eco-enzymes* alone did not show a significant effect on root fresh weight variables. E2 concentration produced the best root fresh weight 35.24 g, which was significantly different from E1 with an average at 29.61 g. Mikofer dose showed a significant effect on root fresh weight variables. The M2 dose produced the best root fresh weight at 39.56 g which was significantly different from M0 (control) with a root fresh weight at 20.63 g (Table 2).

3.4. Root Dry Weight (g)

There was a significant interaction between the concentration of eco-enzyme and the dose of micofer on the root dry weight variable. The best root dry weight was obtained in the E0M2 treatment interaction with an average root dry weight of 11.96 g, while the lowest fresh weight was in the E0M0 treatment interaction with an average root fresh weight of 2.06 g.

The concentration *eco-enzyme* alone did not show a significant effect on root dry weight variables. The concentration of E1 produced the best root fresh weight, which was 8.57 g, which was significantly different
from E0 with an average root dry weight of 7.86 g. Mikofer dose alone showed a significant effect on root fresh weight variables. The M2 dose produced the best root dry weight, which was 9.96 g, which was significantly different from M0 (control) with a root fresh weight of 5.81 g.

3.5. Mycorrhizal Infection (%)

The concentration *of eco-enzyme* alone did not show a significant effect on mycorrhizal infection variables. E1 concentration resulted in the highest mycorrhizal infection with 50.00% infection and the lowest value at E0 with 45.55% infection. A single dose of micofer has shown a significant effect on mycorrhizal colonization in sacha inchi roots (see Fig. 3).

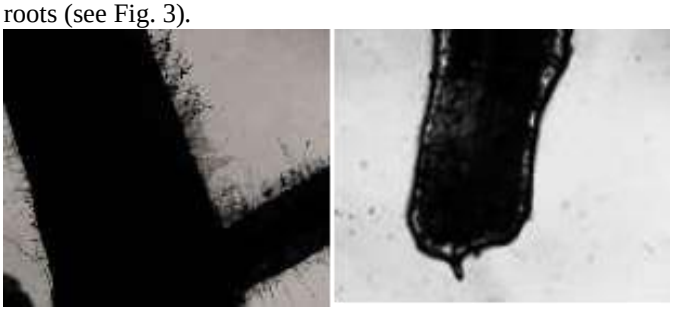

Figure 3 A.Colonization B. No colonization

Application of AMF 40g/plant resulted in the highest mycorrhizal colonization of 64.44% which was categorized as an infection, which was significantly different from M0 (control) with an infection rate of 18.88% (Table 4).

Table 2. Root length, root fresh weight, root dry weight and mycorrhizal infection due to treatment of eco -enzyme concentrations and mycorrhizal applications.

Treatments	Root Length(cm)	Root Fresh Weight (g)	Root Dry Weight (g)	Mycorrhizal Infection (%)
Eco-enzyme (E)				
E0 (0 ml/l)	26.25 (6.76) a	29.61 (5.35) a	7.86 (2.74) a	45.55 a
E1 (22,5 ml/l)	49.85 (6.99) a	28.47 (5.17) a	8.57 (2.98) a	50.00 a
E2 (30 ml/l)	51.55 (7.03) a	35.24 (5.93) a	7.99 (2.89) a	47.77 a
Mycorrhizal (M)				
M0 (0 g/tnm)	44.16 (6.68) b	20.63 (4.40) b	5.81 (2.41) b	18.88 b
M1 (30 g/tnm)	48.19 (6.87) ab	33.13 (5.75) a	8.66 (3.01) a	60.00 a
M2 (40 g/tnm)	55.30 (7.23) a	39.56 (6.30) a	9.96 (3.19) a	64.44 a

Note: The same letter in the same column shows no significant difference according to the DMRT test (α =0.05).

Application mycorrhiza 5 g/plant shows a significant difference in the number of roots in cuttings of patchouli plants (Bancin, 2019). The results of Pratama *et al*. (2019) showed that the treatment of arbuscular mycorrhizal fungi (AMF) 10 g/plant had the best effect on the number of leaves of red bean plants aged 35, 40 and 45 days after planting, leaf area, plant dry weight, number of seeds per plant and seed yield. wet per plot.

The application of eco-enzyme 22.5 ml/l affected the length of sacha inchi plants, this is presumably due to eco-enzymes containing macro elements potassium (K) and phosphorus (P). The availability of nutrients can increase the growth of sacha inchi. [6]Yuliandewi *et al.,* (2018), stated that *eco-enzyme* contains potassium (K) of 203 mg/l and phosphorus (P) of 21.79 mg/l.

The function of Kalium is to increase the intensity of photosynthesis which is can increase the photosynthate content and will increase the growth rate of plants [12]Rahmawan *et al*., 2019. Furthermore, [13]Nurhayati (2021), reported element K is essential in photosynthesis which is involved in ATP synthesis, production in the activity of photosynthetic enzymes (such as RuBP carboxylase), CO2 absorption through the mouth of the leaf, and maintaining electrical balance during photophosphorylation in the chloroplast. Eco-enzymes contain phosphorus that is needed for cell regeneration whether in the vegetative or generative stage. As [14]Safrizal (2014), mentioned that phosphorus plays an important role in photosynthetic activity because it is related to carbohydrate content as a source of energy for plant growth and development.
Mycorrhizal treatment showed a very significant effect on all the variables of plant length and number of leaves. This is because in plants infected with mycorrhiza there are hyphae which function as absorbers of nutrients such as phosphorus. This is in line with the explanation of [15]Bussa *et al.,* (2019), that the main function of the hyphae in mycorrhizal fungi is to absorb phosphorus in the soil. Phosphorus in the soil can be absorbed by roots because roots infected by fungal hyphae in mycorrhizae secrete *phosphatase* which are able to release P from specific bonds, making it available to plants [16]Basri, 2018. The element of phosphorus that is absorbed optimally can be increase the growth rate and yield plant.

Root length showed a significant effect due to micopher. The roots of sacha inchi plants with mycophere treatment were longer than those of the control treatment, this was due to the roots infected with mycopheres resulting in a wider root zone. This is in accordance with [17]Rosnina *et al.,* (2021), that the roots of plants infected with mycorrhizae can expand the root zone so that they can reach the presence of nutrients and increase the absorption of macro nutrients, especially P elements and some micro nutrients. Correlation of the width of the root zone corresponds to the length of the roots of the sacha inchi plant, where the wide root zone will cause the roots of the sacha inchi plant to also have a long size due to mycorrhizal colonization.

There was a very significant difference in root fresh weight after being given a micopher. It is possibility that the roots infected with mycorrhizae can be optimalization the absorbtion of water for photosynthesis and available nutrients such as N, P, K in the soil. This is in line with the statement of [18]Idris *et al.,* (2018), that the high fresh weight of roots is probably due to the nutrient content and N, P, K content at high doses of the planting medium composition. In addition, due to mycoza infection in sacha inchi plants, it causes an expansion of the root zone on plant roots, a wide root zone causes a larger root size and weight compared to roots that are not infected with mycorrhizal.

The root dry weight variable on mycorrhizal administration had a very significant effect, this could happen that the high root dry weight due to mycorrhizal treatment was caused by sufficient nutrient conditions and metabolic

activity that occurred in the sacha inchi plant itself. [18]Idris *et al.*, (2018), stated that metabolic processes and high cell activity will increase root biomass and will affect root dry weight.

Application of mycorrhizal in this study showed a very significant effect on root colonization of sacha inchi. Besides of that to increase absorbtion of nutrients, the colonization mycorrhizal also make the roots zone become wider. This is in line with [17]Rosnina *et al.*, (2021), state that the presence of mycorrhiza can expand the root zone of plants that experience mycorrhizal hyphae colonization so that they can absorb nutrients optimally, especially bound of Phosphore nutrients to become available to plants. Optimization of Phosphore absorption, the process of photosynthesis, respiration, transfer, energy storage, cell division, and enlargement as well as processes in plants can occur optimally [19]Dahlia and Setiono, 2020.

Interaction between the concentration of eco-enzyme and the dose of mycorrhyzae had a very significant effect on the variables of plant fresh weight and plant dry weight. In addition, the eco-enzyme significant effect on the number of leaves at 40 days after planting, stem diameter at 20 and 40 days after planting, and root length.The possibility of the important role of microorganisms in *eco-enzymes* accelerates the decomposition of organic matter and the macro-nutrient content of Phosphorus and Potassium in the soil can be absorbed by external hyphae from plant roots which are colonized by mycorrhizal fungi.

To differentiate the number of leaves, root length, fresh weight of roots, and dry weight of roots from the interaction of application both eco-enzymes and fungi mycorrhizae on plants have been proven that the performance of eco-enzymes as a provide of Phosphore and Kalium better compared than a plant without application. Colonization of mycorrhizae hyphae increases the activity of roots intakes of nutrients and water intake better than in marginal land which is a lack of water.

The existence both of eco enzyme and mycorrhizae application existence proves to increase the number of leaves, increasing the size of the stem diameter and root length of the sacha inchi plant.Plants need nutrients in their growth, these nutrients such as macro nutrients N, P, and K. Plants need these nutrients for the process of plant growth. *Eco-enzyme* itself contains the macro elements potassium (K) of 203 mg/l and phosphorus (P) of 21.79 mg/l [6]Yuliandewi *et al.*, 2018.

With the presence of microorganisms, nutrients and enzymes contained in *eco-* as a result of the eco- fermentation process of fruit waste, it can increase nutrient uptake optimally. The use of biological agents of arbuscular mycorrhizal fungi can increase the ability of plants to take up nutrients (N, K, Mg, Ca, O, H, C, and S), especially phosphorus [20]Zuroidah, 2011.

Utilization of organic matter and enzymes as well as the presence of mycorrhizal hyphae can increase the suitability of sub-optimal land into productive land which can increase the quantity and quality of production of sugar cane and sacha inchi which can be raw materials in producing renewable energy.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusion

- Application of *eco-enzyme* 22.5 ml/l and mikofer 40 g/plant is the best doses to increases the growth rate of sacha inchi cultivated in Inceptisol soils.
- The eco enzyme and mikorizhae were impact to the high of plant, root fresh weight, root dry weight, infection and root length Sacha inchi.

4.2. Recommendation

Eco enzymes and micofer recommended for application to increase the soil fertility and suitability of sub-optimal land.

ACKNOWLEDGEMENT

The authors would like to thank Malikussaleh University for grant No 11/UN45.3.8/HK.02.03/2022 Proyek Advanced Knowledge and Skills for Sustainable Growth Project in Indonesia-Asian Development Bank (AKSI- ADB) Universitas Malikussaleh 2022.

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