Increasing of Aceh's patchouli production with technology of bio-fertilizer local specific mycorrhizal strains in Entisols

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Abstract. Aceh’s patchouli is one of the superior local commodities, and its growth and yield could be improved in various means. One way to increase the patchouli production can be done by using bio-fertilizer mycorrhizal local strain. The purpose of this research was to study effect of bio-fertilizer mycorrhizal local specific strain on the growth and yield of patchouli in Entisols. We conducted two experiment. The first experiment was the propagation technique of mycorrhizae on Entisols soil and the second experiment the test of the fertilizer on a greenhouse pot scale for the growth of patchouli seedlings. The research was arranged by a non-factorial randomized block design with three replications. The investigated factor was the bio-fertilizer mycorrhizal from local specific strain with four levels, i.e. M₀ (without mycorrhizae), M₁ (Glomus mosseae), M₂ (Gigaspora sp) and M₃ (Mixing between Glomus mosseae and Gigaspora sp). The variety of patchouli used in this study was the Tapak Tuan variety. The observed parameters were seedling height and seedling diameter of patchouli at 45, 60 days after planting; additionally the parameter of patchouli yields consist of number of leaves, number of branches and weight of leaves were observed at 90 days after planting, and root colonization by mycorrhizae. The results showed that mixed mycorrhizal strains between Glomus mosseae + Gigaspora sp contributed to the best marks for increasing growth and yield of seedling patchouli in Entisols. The similar trend result was also found in the root colonization by mycorrhizae. The strain of mycorrhizal of Gigaspora sp application was not effective for increasing the growth and yield of Aceh’s patchouli in Entisols.

1. Introduction
Patchouli (Pogestemon cablin Benth.) known as one of the leading plantation commodities that are widely cultivated by the community. As one of the producers of essential oils, patchouli is very necessary for the world of biopharmaca, cosmetics and perfume industry. Currently around 90% of the world's patchouli oil produced by Indonesia comes from Aceh. Besides being used as an ingredient for perfume, Aceh patchouli is also good for aromatherapy, treating diabetic wounds, smoothing the skin, anti-aging, preventing hair loss, anti-bacterial and disinfecting. Patchouli is one of the plantation crops that drives the community's economy. In addition, patchouli has a bright prospect and one of Aceh's mainstay commodities which is widely cultivated in Aceh Besar, Aceh Jaya, North Aceh, South Aceh, West Aceh and all other parts of Aceh. Patchouli productivity and quality is greatly influenced by genetic and environmental factors. Organic patchouli is needed for the perfume, cosmetics and
biopharmaca industries because it is safer and healthier. Besides that, the quality of patchouli oil produced from organic patchouli is very high [1].

Especially in Aceh society generally cultivate patchouli on Ultisol, Inceptisol and Entisols. On moderate to coastal area such as Aceh Besar, South Aceh, West Aceh and North Aceh patchouli production is carried out on Entisols soil. According to Syafruddin et al. [2] utilization of Entisols 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, Entisols 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. 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 [3] [2].

The main obstacle in the utilization of Entisols is low pH and low nutrient content that can be utilized by plants. Measures to increase soil fertility using biological fertilizers need to be done. One of the famous biological fertilizer is mycorrhizae. Mycorrhizae helps the absorption of nutrients needed by plants, especially the elements N and P, while plants can provide carbon elements needed by fungi for survival [2] [4]. Mycorrhizae are found in almost all host plants, including food crops, horticulture and plantations. Mycorrhiza can play a role as bio-fertilizer, bio-protector and bio-regulator which makes it a sustainable environment biological agent [5] [6].

Several studies have shown that patchouli which is given a better growth. Besides that, the problem of drought on land can be overcome for the growth of patchouli plants and crops in line with the use of mycorrhizal bio-fertilizers. The presence of mycorrhizae in the root can increase tolerance to drought stress. Generally the highest leaf alcohol patchouli content is found in the combination of mycorrhizal application with high drought stress [7].

There are several reports that confirm the inoculation of mycorrhizae in patchouli plants from several existing strains. The choice of the right strain type determines the production of patchouli especially organic patchouli. Selection of several FMA fungi for symbiotic responses, such as Glomus etunicatum were identified as the best mycorrhizal symbionts to enhance growth and uptake of P nutrients for patchouli [8]. The type of mycorrhiza used also largely determines the yield of plants including patchouli. This is due to the ability of the mycorrhizal roots to absorb nutrients and protect plants from attack by pathogens, drought and other extreme conditions. [2] [9] [10], helps the absorption of phosphate and nitrogen [11] [12] [13] and hormonal producers such as auxin and gibberellins [14] and able to remediate polluted land [15]. In addition, among the types of mycorrhizae commonly used are Glomus sp., Gigaspora sp., and Acaulospora manihotis [16] [10].

Mycorrhizal fungi also play a role in increasing the number and activity of beneficial soil organisms such as nitrogen solvents and phosphate solvents which are important for the growth of patchouli plants [17]. Plant growth using rhizomicroorganisms (PGPRs) and mycorrhizal fungi have received a lot of attention in recent years. PGPRs known to increase plant growth by producing growth promoting substances, and suppressing root pathogens. Currently the study is directed at interactions between fungi FMA and PGPRs [18]. Therefore this study was conducted to understand the interaction between FMA fungi and their effect on the growth and yield of organic patchouli. According to Syafruddin [19] factors that cause differences in patchouli oil production are physical properties, chemical properties, climate or land character such as altitude, slope, rock conditions above the land surface and others.

Another problem with some varieties of patchouli plants is the susceptibility of these plants to disease attacks, so that mycorrhizae can be bio-protectors to increase patchouli production. Fertilization measures, especially with the application of mycorrhizal fertilizers can increase the potential chemical, physical and biological properties of Entisols soil. Mycorrhizae will work effectively on nutrient-poor soils (marginal) and can help absorb P, N and K effectively so they are available to plants. The presence of mycorrhizae for the availability of nutrients N, P and K in the soil, including Entisols is absolutely necessary [20] [21] [16].
The long-term goal of this study is to produce mycorrhizal biofertilizers as biofertilizers and bioprotectors for increasing production of IPR / Patent-based patchouli. Specifically, this study also investigated the level of mycorrhizal infection of roots, growth and yield of seedling patchouli due to application of mycorrhizal bio-fertilizer produced with various types of mycorrhizal starter.

2. Materials and Methods

2.1 Research Scope

This research was conducted for two years from 2019 until 2020. The first year of research included the propagation technique of mycorrhizae on Entisols soil and the test of the fertilizer on a greenhouse pot scale for the growth of patchouli seedlings. While the second year of research, the adoption of mycorrhizal bio-fertilizer innovations produced in the first year was applied to the patchouli farmer land in Aceh Besar, Aceh Province (2020).

2.2 Materials and Tools

The materials and tools used in this study was patchouli variety of Tapak Tuan. Inoculum AMF strain were Glomus mosseae, Gigaspora sp and mix both with zeolite rock carrier. Nitrogen base fertilizer (Urea), Phosphorous (SP-36) and potassium (KCl) ½ recommended dosage, corn seeds, zeolite, tillage equipment (hand tractor), analytical scales, scrap, spray, oven, sifter, bucket, paint, board, envelope A4, stereo microscope, meter, wood, plastic sack, envelope A4, microscopes, and root recipients of the Win-Rhizo Program.

2.3 Research Design

Experiment 1 was an experiment to produce mycorrhizal bio-fertilizer in Entisols soil for the production of organic patchouli. Propagation techniques using corn host plants. In the process of propagation carried out maintenance, topping and stressing. Mycorrhizal harvesting is done after the host plant is 75 days old. The type of mycorrhizae that were tried consisted of Glomus mosseae, Gigaspora sp. and mixed Glomus mosseae + Gigaspora sp. (1:1) which is applied to the variety patchouli Tapak Tuan.

The parameters in this study were the first seedling growth components observed included seedling height, seedling diameter at 45 and 60 days after planting (DAP); additionally the parameter of seedling patchouli yields consist of number of leaves, number of branches and weight of leaves were observed at 90 days after planting, and root colonization by mycorrhizae. Mycorrhizal colonization or the degree of AMF infection in host plant roots was seen through the coloring method Kormanik and Graw (1982) [22] and Syafruddin et al, (2010) [23].

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 LSD test at level 5% (p < 0,05 LSD Test).

3. Result and Discussion

Experiments 1. Mycorrhizal Bio-fertilizer Propagation Techniques

Experiment 1 has been successfully carried out and the output is the availability of mycorrhizal biofertilizers of the type Glomus mosseae, Gigaspora sp., and mixed Glomus mosseae + Gigaspora. The degree of root infection in mycorrhizal bio-fertilizer obtained is presented in Table 1.
**Table 1. Mycorrhizal bio-fertilizers and root infection rates.**

| No | Type of Mycorrhizal Bio-fertilizer Starter | Infection rate FMA (%) | Criteria | Rajapakse and Miller (1992) |
|----|------------------------------------------|-------------------------|----------|----------------------------|
| 1  | *Glomus mosseae*                         | 85 %                    | Very high|                            |
| 2  | *Gigaspora*                              | 70 %                    | High     |                            |
| 3  | Mixed *Glomus + Gigaspora*               | 90 %                    | Very high|                            |

Source: Laboratory Analysis (2019)

Based on Table 1, the highest level of mycorrhizal infection was obtained in the type of mycorrhizal mixed between *Glomus mosseae + Gigaspora sp*. Furthermore, Rajapakse and Miller [24] categorize the degree of infection FMA that percentage 0 – 5 class category 1 (very low), 6 – 25 % class category 2 (low), 26 – 50 % class category 3 (medium), 51 - 75 % class category 4 (high) and 75 – 100% class category 5 (very high). This happens because the results of root biomass and plant crowns are indeed more common in mixed species *Glomus mosseae + Gigaspora sp* compared to separate species *Glomus mosseae* and *Gigaspora sp*. Syafruddin et al. [25] and Syafruddin et al. [2] reported that mycorrhizae were mixed *Glomus mosseae + Gigaspora sp* usually have good adaptability and growth in the polluted and tropical regions. Experiments on mixed type mycorrhizal corn starter plant host *Glomus mosseae + Gigaspora sp* provides very high root biomass compared to a separate starter from *Glomus mosseae* and *Gigaspora sp*, and if mixed both will be able to synergize properly spur plant growth. Next Safrianto et. al., [26] get that the growth of the roots of the chili plants that are given mixed mycorrhizal fertilizer *Glomus mosseae + Gigaspora sp* tend to be better compared to separate types *Glomus mosseae* and *Gigaspora sp*. Mycorrhizal bio-fertilizers produced are very effective and have succeeded in increasing the growth of organic patchouli seed production. Seedling growth on average increases to 50 – 75 %.

In addition to the type of mycorrhizal starter, the environmental condition of mycorrhizal bio-fertilizer propagation also greatly determines the success rate of mycorrhizal propagation. The environmental conditions of mycorrhizal propagation indicate a very high level of root infection power, especially in species *Glomus mosseae, Gigaspora sp* and mixing *Glomus mosseae + Gigaspora sp*. Next [16] showed that soil pH, temperature, humidity and soil type determine the success rate of mycorrhizal propagation. The type of strain from mycorrhizal bio-fertilizers also determines the success rate of mycorrhizal infection power. Previous research [2] also found seedling growth and viability and patchouli seed vigor given mycorrhizal bio-fertilizer increased.

**Experiments 2. Patchouli Seedling Growth Applied by Mycorrhizal Bio-fertilizer**

Average seedling growth, yield and root colonization of seedling patchouli applied by mycorrhizal bio-fertilizer are shown in Table 2.

Patchouli seedlings produced from the application of mycorrhizal bio-fertilizers produced prove that the best seedling growth and seed quality are found in the use of mixed strains (*Glomus mosseae + Gigaspora sp*). Then followed by strain *Glomus mosseae*, and *Gigaspora sp*. The performance of patchouli seedlings given mycorrhizae at the time of nursery looks good. Provision of mycorrhizae at the time of nursery results in good plant performance. That is because the earlier mycorrhizae are given it will affect the rate of root infection by mycorrhizae. In general, many plants have higher levels of mycorrhizal infection during vegetative growth [12] [6].
Table 2. Average seedling growth, yield and root colonization of seedling patchouli applied by mycorrhizal bio-fertilizer

| Parameters                          | Strain of mycorrhizae | LSD_{0.05} |
|-------------------------------------|-----------------------|------------|
|                                     | M0   | M1   | M2   | M3   |          |
| Seedling height at 30 DAP (cm)      | 21.00 \textsuperscript{a} | 30.67 \textsuperscript{c} | 24.33 \textsuperscript{b} | 42.33 \textsuperscript{d} | 3.19     |
| Seedling height at 60 DAP (cm)      | 25.33 \textsuperscript{a} | 37.33 \textsuperscript{b} | 26.33 \textsuperscript{a} | 52.67 \textsuperscript{c} | 2.88     |
| Seedling diameter at 30 DAP (mm)    | 2.93 \textsuperscript{a}  | 3.47 \textsuperscript{a}  | 4.50 \textsuperscript{b}  | 6.67 \textsuperscript{c}  | 0.69     |
| Seedling diameter at 60 DAP (mm)    | 3.27 \textsuperscript{a}  | 6.50 \textsuperscript{c}  | 5.47 \textsuperscript{b}  | 8.67 \textsuperscript{d}  | 1.16     |
| Number of leaves at 90 DAP (leaves)| 42.33 \textsuperscript{a} | 135.00 \textsuperscript{c} | 78.33 \textsuperscript{b} | 257.00 \textsuperscript{d} | 7.70     |
| Number of branches at 90 DAP (branches)| 11.67 \textsuperscript{a} | 12.00 \textsuperscript{a} | 18.33 \textsuperscript{b} | 26.00 \textsuperscript{c} | 2.56     |
| Leaves weight at 90 DAP (g)         | 3.30 \textsuperscript{a}  | 4.34 \textsuperscript{c}  | 3.57 \textsuperscript{b}  | 8.48 \textsuperscript{d}  | 0.74     |
| Root colonization (%)               | 1.28 \textsuperscript{a}  | 64.66 \textsuperscript{c} | 58.95 \textsuperscript{b} | 77.54 \textsuperscript{d} | 3.32     |

Note: Value followed by the same letter, the same rows is not significantly different at LSD test (p 0.05); DAP day after planting.

Patchouli seedling growth and yield of all parameters tested in the field up to 30, 60 and 90 DAP was very good for mixed species *Glomus mosseae* + *Gigaspora sp*. There were no significant obstacles in testing pot experiments in the greenhouse for the growth of patchouli seedlings. The indicator can be seen from the seedling height, seedling diameter, number of leaves and the number of branches formed in variety tested. This is in line with research [8] and [2] and [3] which examines the growth of patchouli with mycorrhizae. The advantages of mixed mycorrhizae for the growth of horticultural crops such as chili have also been studied by [18].

Conclusion

Propagation techniques for mycorrhizal bio-fertilizer have been carried out to increase the production of seed values with the best strains obtained by mixed mycorrhizae *Glomus mosseae* + *Gigaspora sp* with 90% root colonization rate (very high category). Giving mycorrhizae in patchouli seeds increase seedling growth of 50-70%. The best of strain mycorrhiza for all parameter was mixing *Glomus mosseae* + *Gigaspora sp*. Further testing is needed on patchouli land using a strain of mycorrhizal bio-fertilizer that has been produced. Field research on Entisols soil is very necessary in the second year.

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