

The effect of biochar and liquid organic fertilizer to *Mentha spicata* physiological activities

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Abstract. Mint (*Mentha spicata* L.) is a medicinal plant that produces essential oils usually used in medicines, food, toothpaste, and cosmetics. Mint leaves are the primary organ taken as an essential oil production material. Increasing leaf production, which is studied through the physiological activity of mint, can be done by meeting plant nutrient needs. Biochar and liquid organic fertilizer (LOF) can be used as alternative soil conditioners and fertilizers to meet plant nutrient needs. This research was conducted in the Faculty of Agriculture's Greenhouse using a factorial Randomized Complete Group Design (RCGD), which consists of two factors, namely biochar and liquid organic fertilizer of fish waste. The type of biochar consists of no biochar treatment, palm fiber biochar, and rice husk biochar. Fish waste liquid organic fertilizer consists of no LOF treatment, 5 ml/L water, 10 ml/L water, and P3 15 ml/L water. The results of biochar types increased the chlorophyll content of mint, which were 1.14, 0.51, and 1.14 mg.g⁻¹. The treatment of fish waste liquid organic fertilizer increased the transpiration rate of mint, which were 1.27 CO₂⁻¹.L⁻¹.minute⁻¹.

1 Introduction

Mint is a medicinal plant that is widely utilized as a source of essential oil. Mint leaves contain essential oils that are utilized in the food, pharmaceutical, cosmetic, and healthcare industries [1]. Mint offers a variety of benefits and has a high market value, making it a potentially profitable crop [2]. However, a key challenge in mint cultivation is the lack of accurate calculations for the optimal dosage of fertilizers and nutrients required to promote healthy growth. Mint plants need sufficient nutrients to produce optimal growth. In addition to nutrients, fertile planting media also has an important role in the growth of mint plants. The use of liquid organic fertilizer (LOF) derived from fish waste and soil improver in the form of biochar has the potential to increase mint growth and reduce organic waste in the community.

Fish waste is one of the wastes generated from the fish processing industry. About 25-35% of fish production becomes waste due to inadequate technology, storage facilities, and fish processing in Indonesia [3]. Use of fish waste as liquid organic fertilizer (LOF) in

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addition to optimizing waste management also has the capacity to enhance plant growth [4], one of which is mint. Liquid organic fertilizer is useful for increasing the formation of leaf chlorophyll, thereby increasing the photosynthetic ability of plants and the absorption of nitrogen from the air [5]. The application of liquid organic fertilizer can enhance plant vigor so that plants become sturdy and strong [6]. It also boosts their resistance to drought, environmental stress, and attacks from disease causing pathogens, while promoting overall plant growth [7].

Rice husks and palm fibers are by-products of agricultural activities and the palm flour industry. These organic materials can be processed into biochar, which serves as an effective soil improver. Palm fiber contains agricultural and forestry residual biomass so it has the potential to be used as biochar [8]. Rice husk biochar is highly adsorptive and possesses a significant nutrient retention capacity along with a high silica content, which enhances soil fertility and improves the effectiveness of fertilizers when combined [9]. The elevated silica content in rice husk biochar contributes nutrient retention, turgidity, and overall plant growth [10]. The use of biochar as a soil amendment has the potential to increase the growth of mint plants.

Plant physiological processes have an important effect on plant growth. Mint growth in the form of number of leaves, plant height, and number of branches is influenced by plant physiological activities. Physiological response as a form of plant adaptation to environmental conditions is related to plant productivity due to changes in physiological mechanisms which then become the key to the phenotype and productivity produced by plants [11]. Increased plant physiological activity can be stimulated by the use of LOF and soil amendments. This study aims to determine the interaction, type of biochar, and the appropriate concentration of LOF to increase the growth and physiological activity of mint.

2 Materials and Methods

Experimental research was conducted in Greenhouse A, Faculty of Agriculture, Sebelas Maret University Surakarta, at coordinates 7°33'41.7" LS and 110°51'32.6" East with an altitude of 96 meters above sea level (masl). The research was conducted from August to November 2022. The research method used a factorial Randomized Complete Group Design (RCGD) with two factors. The first factor is the type of biochar consisting of 3 types, namely without biochar treatment (B0); palm fiber biochar (B1); rice husk biochar (B2). The second factor is the concentration of fish LOF obtained from Balekambang Fish Market, Surakarta. The types of fish used are diverse. Fish LOF consists of 4 levels, namely without LOF treatment (P0); 5 ml/L water (P1); 10 ml/L water (P2); 15 ml/L water (P3). Each treatment combination was repeated 3 times so there were 36 experimental units. The research data were analyzed using analysis of variance (ANOVA) at the 5% error rate. If significantly different, it will be further tested using DMRT at the 5% level using SPSS *software*.

The research implementation began with the preparation of fish waste LOF and biochar. Biochar materials were dried first before making biochar through the pyrolysis process. Fish waste LOF was dissolved into water according to the treatment. The second step is the preparation of planting media by putting the decomposed soil into polybags with a volume of 25 x 25 x 25 cm. Biochar is then mixed into the soil, which is 12.5 g/polybag each for rice husk biochar and palm fiber biochar. The third step is the preparation of planting material in the form of mint propagation using the cuttings method. Cuttings that are 1 week old and have grown roots are inserted into the polybag. The fourth step is maintenance which includes weeding, watering, and pest control. The fifth step is fish waste LOF treatment which is given to the plants once a week when the plants are 2 weeks old after planting. Observation

variables include transpiration rate, photosynthesis rate, stomatal conductance, number and width of stomatal openings, chlorophyll content, net assimilation rate, and relative growth rate.

3 Result and Discussion

3.1 Transpiration rate

Transpiration is the process of loss of water vapor from living plant tissues located above the soil surface, one of which is through leaf pores such as stomata [12]. Transpiration is a physiological process related to the internal and external conditions of the plant. The higher the transpiration rate indicates that the speed of transport of water and dissolved nutrients in plants is getting higher, and vice versa. The observation of transpiration rate was done when the plants were 12 weeks old. The highest transpiration rate was 1.27 CO₂⁻¹.L⁻¹.minute⁻¹ in the 15 ml/L fish waste LOF. The type of biochar had no significant impact on the transpiration rate, while the concentration of fish waste LOF significantly affected the transpiration rate (Table 1).

Table 1. Effect of biochar type and fish waste LOF concentration on transpiration rate, photosynthesis rate, and stomatal conductance

| Treatment | Transpiration Rate (CO ₂ ⁻¹ .L ⁻¹ .minutes ⁻¹) | Photosynthesis Rate (μmol.CO ₂ .m ⁻² s ⁻¹) | Stomatal Conductance (mol.H ₂ O.m ⁻² s ⁻¹) |
|--|--|---|--|
| Biochar Type | | | |
| No Biochar | 0,51 a | 0,14 a | 2,13 a |
| Palm Fiber | 0,72 a | 0,17 a | 2,45 a |
| Rice Husk | 0,78 a | 0,14 a | 2,11 a |
| Fish Waste LOF Concentration (ml/L) | | | |
| 0 | 0,25 b | 0,14 a | 1,99 a |
| 5 | 0,72 ab | 0,15 a | 2,23 a |
| 10 | 0,44 b | 0,15 a | 2,27 a |
| 15 | 1,27 a | 0,16 a | 2,42 a |

Description: Numbers that have the same letter in each treatment indicate no significant difference according to DMRT at the 5% level.

The application of biochar types that do not significantly affect the transpiration rate is because biochar takes a considerable amount of time to break down in the soil and provide nutrients for plants. Biochar provides N nutrients slowly in the soil [13]. The use of biochar does not supply nutrients right away without undergoing the decomposition process [14]. The release of nutrients from biochar occurs slowly or slow release so that biochar can supply nutrients to plant in the long term. This is in accordance with the research [15] which explains that biochar has specific physiochemical qualities that allow it to be used over a long period of time, storing carbon and improving soil health. The type of biochar that does not significantly affect the transpiration rate also indicates that the palm fiber and rice husk biochar used has not been able to increase the availability of water in the planting media. The availability of sufficient water can increase the transpiration rate of plants.

The use of fish waste LOF had a significant impact on the transpiration rate of mint plants. A concentration of 15 ml/L fish waste LOF resulted in a higher transpiration rate of 1.27 CO₂⁻¹.L⁻¹.minute⁻¹, which was not significantly different from that at concentration of 5 ml/L. Additionally, the application of fish waste LOF can enhance the availability of essential

nutrients such as N, P, and K that plants require [4]. Enhanced availability of nutrients in plants can optimize the opening of stomata so that the transpiration process runs more efficiently. Potassium is an essential element that plays a role in stomatal opening [16]. Stomata that are more widely open will increase stomatal conductivity so as to accelerate the transpiration rate. Open stomata become a link between the intercellular cavity and the atmosphere. If the water vapor pressure in the atmosphere is lower than the intercellular cavity, the water vapor in the cell cavity will escape [17].

3.2 Photosynthesis rate

Photosynthesis is a process that generates energy within the plant. The process of photosynthesis utilizes water and carbon dioxide and the help of sunlight so as to produce oxygen and food substances needed by plants [18]. The type of biochar and fish waste LOF did had no significant impact on the rate of photosynthesis (Table 1). Biochar takes a long time to decompose in the soil. Similar research [19] also explained that the use of organic materials such as straw has slow mineralization properties so that the access of soil microorganisms in decomposition is also hampered. Nutrients produced by biochar are also slow release so they cannot be available quickly. Fish waste LOF treatment in this study also did not significantly affect the photosynthesis rate. This is because the N content of LOF fish waste at 5-15 ml/L concentration has not been able to increase the photosynthesis rate of plants. High N content can increase photosynthesis tolerance to high temperature stress. CO₂ assimilation is generally inhibited in high temperature environments so that assimilation decreases, but high N content can significantly offset high temperature constraints on CO₂ assimilation [20].

External factors that affect the rate of photosynthesis are temperature, air humidity, light intensity, groundwater, soil pH, and CO₂ levels in the environment [21] [22]. Temperature is one of the environmental factors that significantly influences the physiological processes of plants including the rate of photosynthesis. The research was conducted in a greenhouse with an average temperature of 34°C. Temperatures that are too high result in CO₂ levels less soluble in water and chloroplasts thus inhibiting the process of photosynthesis rate [23].

3.3 Stomatal conductance

Stomatal conductance is a response to the opening and closing of stomata which is influenced by the width of the opening and stomatal density. The treatment of biochar type and fish waste LOF concentration had no significant effect on stomatal conductance (Table 1). The provision of biochar and fish waste LOF has not been able to significantly increase stomatal conductance. Stomatal conductance is affected by the availability of water in the growing media [24]. Biochar when used as a soil amendment has the potential to enhance water availability in the growing medium. The more water availability in the soil can increase stomatal conductance, and vice versa. Biochar can hold water in soil with limited water holding capacity [25]. Maximum stomatal conductance makes the transpiration rate also maximum, but a decrease in stomatal conductance inhibits the transpiration rate [26]. Low soil moisture causes plants to lose turgor and then the plants respond by closing the stomata so as not to lose much water which has implications for decreasing stomatal conductance.

3.4 Chlorophyll contents

Chlorophyll is a pigment that gives plants their green color. This pigment is involved in the photosynthesis process of plants by synthesizing and transforming light energy into chemical energy. The type of biochar significantly affected the levels of chlorophyll a, b, and total chlorophyll, while the concentration of fish waste LOF had no significant effect on chlorophyll content (Table 2).

Table 2. Effect of biochar type and fish waste LOF concentration on chlorophyll content

| Treatment | Chlorophyll contents (mg.g ⁻¹) | | |
|--|--|---------------|-------------------|
| Biochar Type | Chlorophyll a | Chlorophyll b | Total Chlorophyll |
| No Biochar | 0,82 b | 0,28 b | 0,82 b |
| Palm Fiber | 1,14 a | 0,51 a | 1,14 a |
| Rice Husk | 0,92 b | 0,36 b | 0,92 b |
| Fish Waste LOF Concentration (ml/L) | | | |
| 0 | 0,88 a | 0,32 a | 0,88 a |
| 5 | 0,92 a | 0,35 a | 0,92 a |
| 10 | 1,05 a | 0,44 a | 1,05 a |
| 15 | 1,00 a | 0,40 a | 1,00 a |

Description: Numbers that have the same letter in each treatment indicate no significant difference according to DMRT at the 5% level.

The type of palm fiber biochar result the highest levels of chlorophyll a, b, and total chlorophyll, which were 1.14, 0.51, and 1.14 mg.g⁻¹, respectively. Palm fiber biochar is able to meet the needs of N elements in plants so that it has a significant effect on chlorophyll content. The application of biochar can enhance soil fertility by increasing pH, CEC, organic C, and meeting the needs of nitrogen [27]. Nitrogen acts as the main nutrient for chlorophyll [28]. The high nitrogen content makes the leaves greener and more durable because it has a high chlorophyll content. Biochar has a large specific surface area, large pore volume, and high cation exchange capacity so that it can increase the absorption of water and nutrients [29]. LOF treatment of fish waste did not have a significant effect on chlorophyll content. This is because the concentration of fish waste LOF has not been sufficient to fulfill the nutrient requirements of plants.

3.5 Number of stomata

Stomata are a modification of epidermal tissue found on the leaf surface. Stomata play a role in regulating the exchange of O₂, CO₂, and water vapor in plants. The number of stomata in plants varies, influenced by internal and external factors. Biochar type and LOF concentration had no significant impact on the number of stomata (Table 3).

Table 3. Effect of biochar type and fish waste LOF concentration on the number and width of stomatal openings

| Treatment | Number of stomata (pieces) | Stomatal opening width (µm) |
|--|----------------------------|-----------------------------|
| Biochar Type | | |
| No Biochar | 201,33 a | 6,38 a |
| Palm Fiber | 203,75 a | 6,19 a |
| Rice Husk | 206,92 a | 6,03 a |
| Fish Waste LOF Concentration (ml/L) | | |
| 0 | 204,33 a | 6,11 a |
| 5 | 206,89 a | 6,06 a |
| 10 | 202,56 a | 6,19 a |
| 15 | 202,22 a | 6,44 a |

Description: Numbers that have the same letter in each treatment indicate no significant difference according to DMRT at the 5% level.

The provision of biochar and fish waste LOF that has no effect on the number of stomata is caused by external factors outside the plant. The number of stomata besides being influenced by internal factors is also influenced by external factors, namely environmental conditions such as light intensity, soil pH, and air temperature [30]. Temperature is an environmental factor that has an important effect on the number of stomata [31]. Air temperature conditions in the research environment that were too hot resulted in drought stress in mint plants. Increased air temperature is one of the impacts of global warming. The higher the light intensity and air temperature causes the number of stomata to also increase, and vice versa [32]. Observations of the number of stomata were made between 09.00-11.00. The afternoon observation time resulted in the air temperature at the experimental location becoming higher. High temperatures cause an increase in the rate of plant respiration so that the concentration of CO₂ in the leaves increases. Increased CO₂ levels result in fewer stomata [33].

3.6 Stomatal opening width

Stomata are oval-shaped slits found in epidermal tissue. Stomata are flanked by two guard cells that regulate the gap or width of the stomatal opening. The width of stomatal openings is related to the rate of absorption of CO₂ from the air which is used as raw material in the photosynthesis process [34]. The treatment of biochar type and fish waste LOF concentration had no significant effect on the width of stomatal openings (Table 3). The width of stomatal openings is influenced by several factors, one of which is the number of stomata. A greater number of stomata correlates with increased stomatal density, but results in a narrower stomatal opening width [35]. In addition, the width of stomatal openings is also affected by the availability of water in plants. The use of this type of biochar has not been effective in retaining and enhancing the availability of water required by plants, which means it does not influence the width of stomatal openings. Plants that experience drought stress show a physiological response in the form of stomatal opening [36].

3.7 Net assimilation rate

Net assimilation rate is a parameter used to measure the ability of photosynthesis to produce plant dry matter. Net assimilation rate is defined as the increase in dry weight per unit of leaf

area over time. The type of biochar the concentration of fish waste LOF did not have a significant impact on the net assimilation rate (Table 4).

Table 4. Effect of biochar type and fish waste LOF concentration on net assimilation rate and relative growth rate

| Treatment | Net Assimilation Rate (g ⁻¹ .m ⁻² .week ⁻¹) | | Relative Growth Rate (g ⁻¹ .m ⁻² .week ⁻¹) | |
|--|--|----------|---|----------|
| | 4-8 MST | 8-12 MST | 4-8 MST | 8-12 MST |
| Biochar Type | | | | |
| No Biochar | 0,0042 a | 0,0019 a | 0,297 a | 0,118 a |
| Palm Fiber | 0,0038 a | 0,0021 a | 0,257 a | 0,130 a |
| Rice Husk | 0,0036 a | 0,0020 a | 0,298 a | 0,077 a |
| Fish Waste LOF Concentration (ml/L) | | | | |
| 0 | 0,0039 a | 0,0020 a | 0,306 a | 0,091 a |
| 5 | 0,0038 a | 0,0020 a | 0,308 a | 0,117 a |
| 10 | 0,0041 a | 0,0022 a | 0,247 a | 0,121 a |
| 15 | 0,0037 a | 0,0017 a | 0,274 a | 0,102 a |

Description: Numbers that have the same letter in each treatment indicate no significant difference according to DMRT at the 5% level.

The variety of biochar and the level concentration of fish waste LOF had no effect on the net assimilation rate because the treatment was unable to supply sufficient nutrients to plants. The use of biochar can enhance the availability of nutrients in the soil, but in a long time. The element nitrogen is important for the process of cell division [37]. The adequacy of nitrogen absorbed by plants affects the weight of dry biomass of plants [38]. Another factor that affects NAR is the number of active leaves that are not shaded. The small number of leaves, relatively small leaf size so as not to shade the leaves below, makes almost all leaves still effective in photosynthesizing. NAR is also influenced by leaf area [39]. An increase in leaf area does not always result in a higher net assimilation rate. This is due to some leaves being shaded, which prevents them from performing photosynthesis effectively [40]. The net assimilation rate is affected by leaf area and plant dry weight. As the leaf area and dry weight of the plant increase, the net assimilation rate also rises, and vice versa.

3.8 Relative growth rate

Relative growth rate is a parameter used to measure changes in plant weight over a specific duration of time. The type of biochar type and the concentration of fish waste LOF did not have a significant impact on the relative growth rate (Table 4). Giving biochar or fish waste LOF has not been able to increase relative growth rate because biochar has not been effective in retaining the water required by plants in the soil, leading to drought stress in mint [41]. Too high temperatures cause plants to experience drought stress so that plants experience stress which has an impact on reducing plant productivity [42]. These results are in accordance with research [43] that the treatment of rice husk biochar doses with less than optimal research environmental conditions (drought stress) makes LOF treatment unable to significantly increase the relative growth rate of kale.

4 Conclusion

Climate change has a various effect on plant physiological activity. According to the results of this study, the provision of several types of biochar and fish waste LOF has not been able

to increase the physiological activities of mint plants which include photosynthetic rate, stomatal conductance, number of stomata, stomatal opening width, net assimilation rate, and relative growth rate. The application of palm fiber biochar can increase the levels of chlorophyll a, b, and total chlorophyll in mint plants. The use of fish waste LOF at concentration of 15 ml/L can enhance the transpiration rate of mint plants. Further research needs to be done to find out more about the effect of using palm fiber biochar and fish waste LOF on mint plants.

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