International Journal of Agriculture System
Vol. 13 Issue 1, June 2025
Nationally Accredited Journal Decree No. 177/E/KPT/2024
P-ISSN: 2337-9782, E-ISSN: 2580-6815. **DOI: 10.20956/ijas.v13i1.5504**

The Effect of Providing Sugarcane Bagasse Biochar and Moringa Leaf Local Microorganism (LMO) on the Growth of Sugarcane Seedlings (*Saccharum officinarum* Linn)

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How to Cite: Kasim, N., Y. Musa, N.A. Putri. (2024). The Effect of Providing Sugarcane Bagasse Biochar and Moringa Leaf Local Microorganism (LMO) on the Growth of Sugarcane Seedlings (*Saccharum officinarum* Linn). *Int. J. Agr. Syst.* 13(1): 1-13.

ABSTRACT

This research aims to study the effect of sugarcane bagasse biochar and Moringa leaf LMO's local microorganisms on sugarcane seedlings' growth. This research was carried out at the experimental garden of the Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, in June - October 2023. This research is arranged in the form of an experiment using a Split Plot Design (SPD), with the main plot being the LMO of Moringa leaves, which consists of 3 levels, namely 0 mL/L water, 200mL/L water, and 400 mL/L water. The subplot is sugarcane bagasse Biochar dosages, which consist of 4 levels, namely 0 g, 50 g, 100 g, and 150 g per plant. Each treatment combination consisted of 3 plant units and was repeated three times, so that there were 108 experimental units. The study results showed no interaction between the LMO concentration of Moringa leaves and the dose of bagasse biochar on all observed parameters. Moringa leaf LMO 200 mL/L water had the best effect on the highest number of tillers. 100 g sugarcane bagasse biochar gave the best impact on the highest chlorophyll levels, the highest chlorophyll b content LMO, and the highest total chlorophyll content, while 150 g bagasse biochar gave the best effect on the highest increase in stem diameter and the highest root fresh weight.

Keywords:
Biochar; local microorganism; sugarcane seeds.

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is a plant that is the main raw material for sugar and is cultivated in tropical climates. This plant is so needed that demand continues to increase along with population growth. To date, sugar cane development has been carried out in Java, North and South Sumatra, and Sulawesi and Nusa Tenggara. Increasing sugar consumption is the main element for growth in new areas, as well as to meet the market in Eastern Indonesia (Directorate General of Agriculture, 2019).

The limited availability of land makes the need land for nurseries increasingly difficult. Seed preparation technology is required, which is short, does not take up space, and is of good quality. The bud set technique is the nursery technique that can produce high-quality seeds and does not require seed preparation through tiered gardens. Bud set is a sugarcane breeding technique obtained from sugarcane stems in single-eyed cuttings with a cutting length of approximately 5 cm and the eye position in the middle of the cutting length (Haqi et al., 2018). Besides expanding the sugarcane plantation area, sugarcane cultivation techniques must also be considered to increase domestic sugar productivity and production.

According to the 2023 Central Statistics Agency, cane sugar production will increase in 2022 by 2.40 million tons. However, domestic sugar production has not balanced the increase in sugar consumption, requiring Indonesia to import sugar from various countries. In 2022, Indonesian sugar imports from abroad will reach 6.01 million tons. One of the reasons why Indonesia is still importing sugar is due to low productivity. The low level of domestic sugar production is due to inappropriate cultivation practices and land fertility levels that continue to decline.

Sugarcane production will increase in line with the increase in sugarcane yield value. Yield is the sugar content contained in sugar cane plants. The decrease in sugarcane yield can be caused by several factors, including the cultivation process, the low quality of seeds, the prevailing climate, and the soil's supply of nutrients. The nutritional needs of sugar cane seeds need to be considered so that sugar cane plants produce maximum results.

Sugarcane bagasse can be used as raw material for biochar. Biochar is a new term used to refer to powdered charcoal, which is a porous material from various biomasses. Sugarcane bagasse is a biomass reported to have relatively large levels of cellulose and hemicellulose, so when pyrolyzed, it will produce a relatively high carbon content, which will benefit plants. Biochar becomes more carbon-containing as the temperature is increased, and the carbon content ranges from 58 to 64% in the temperature range of 300 to 700°C (Irfan et al., 2016). With direct and indirect effects on the properties of contaminated soil, biochar can increase the potential of soil to produce high crop yields and improve soil characteristics (Al-Wabel et al., 2015; Iqbal, 2018). Ardi et al. (2023) indicate that applying 91g/plant of sugarcane bagasse biochar soil gave good results in the growth of sugarcane seedlings. Compared to other materials, biochar is a more environmentally friendly, pollution-free, and renewable material that can be used as an alternative planting medium.

Biochar as a soil amendment is an alternative that can be used for sugarcane cultivation. Biochar can improve soil function and maintain nutrients and their availability in the soil. The correct use of biochar is a crucial first step for the success of sugarcane cultivation, which will ultimately encourage an increase in its productivity. Utilizing sugarcane bagasse waste as a nutrient additive has the potential for biochar (biological charcoal) to overcome several limitations in carbon management. The ability of biochar as a soil amendment will positively correlate with soil fertility and plant productivity (Ferjani et al., 2020).

Apart from providing biochar, adding an activator supplement in the form of LMO from Moringa leaves can be another alternative to support sugarcane cultivation. According to Laepo et al. (2018), giving LMO Moringa leaves with banana peel as much as 600 mL/2L of water significantly affected the height and length of leaves in sweet corn plants. Moringa leaf LMO can help sugarcane seedlings fulfill nutritional to maximize seedling growth so that seedling growth is maximum. In addition, adding LMO to plants can increase the activity of soil microorganisms that are beneficial to plants. Moringa leaf extract contains the hormone cytokinin, which can help plants grow faster (Krisnadi and Dudi, 2012), macro elements such as potassium, calcium, magnesium, sodium, and phosphorus, and microelements such as manganese, zinc, and iron (Supriyadi et al., 2022).

Biochar and LMO from Moringa leaves treatments have different ways of working to increase the growth of sugarcane seedlings. With differences in working methods, nutrient needs can be met properly. Biochar works in the soil by helping improve soil quality and can increase the soil's CEC (cation exchange capacity), minimizing nutrient leaching risk. Biochar accumulates in the soil so that improvements in soil quality are faster and more sustainable. Meanwhile, Moringa leaf LMO is a supplier of growth hormones and can also be an alternative to support plant nutrient needs.

2. Materials and Methods

2.1 Place and time

This research was carried out at the experimental garden of the Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, at an altitude of 12 m above sea level (5◦07'39''S 119◦28'59''E) from June to September 2023.

2.2 Research methods

This research used 20x30 cm polybags with a planting media volume of 7 liters. Each polybag contained one sugarcane seedling.

This research was conducted using a Split Plot Design (SPD). The main plots are various concentrations of Moringa leaf LMO (M), which consist of 3 levels: m0 = Control (Without Moringa leaf LMO), m1 = 200 mL Moringa leaf LMO/L water, m2 = 400 mL Moringa leaf LMO/L water. The subplot is the biochar dosages (B), 4 levels: b0 = Control (Without biochar), b1 = 50 g, b2 = 100 g, b3 = 150 g per plant. Each treatment combination consisted of 3 plant units and was repeated 3 times, so that there were 108 experimental units.

2.3 Observation variables

1. Stem diameter was observed at 4, 7, and 10 WAP (Week After Planting). This observation was made by measuring the plant stem 5 cm from the ground surface using a caliper.
2. Root fresh weight, this observation was made at the end of the study. Plant roots cleaned with water are then weighed using an analytical balance.
3. Number of tillers, calculating the number of tillers is done by counting the number of tillers in each growing plant. This observation was carried out at the end of the research when the plants were 10 WAP.
4. Chlorophyll a, b, and total leaf chlorophyll components were observed using a Content Chlorophyll Meter (CCM 200+). Observations were made on chlorophyll a, chlorophyll b, and total leaf chlorophyll content using the formula: Leaf chlorophyll content = a + b(CCI)c, where a, b, and c are constants, and CCI is the leaf chlorophyll index data read on CCM 200+, where:

Table 2. Chlorophyll constant values a, b, and c

Parameter	y = a + b (CCI) c		
	A	B	C
Chlorophyll a	-421,35	375,02	0,1863
Chlorophyll b	38,23	4,03	0,88
Chlorophyll tot	-283,2	269,96	0,277
A	-3,50	3,96	0,027

Source: Nasaruddin, 2022.

3. Results and Discussion

3.1 Result

3.1.1. Increase in stem diameter (mm)

The analysis of variance showed that the biochar treatment had a significant effect. In contrast, the Moringa leaf LMO treatment and its interactions had no significant impact on the increase in stem diameter of the sugar cane seedlings.

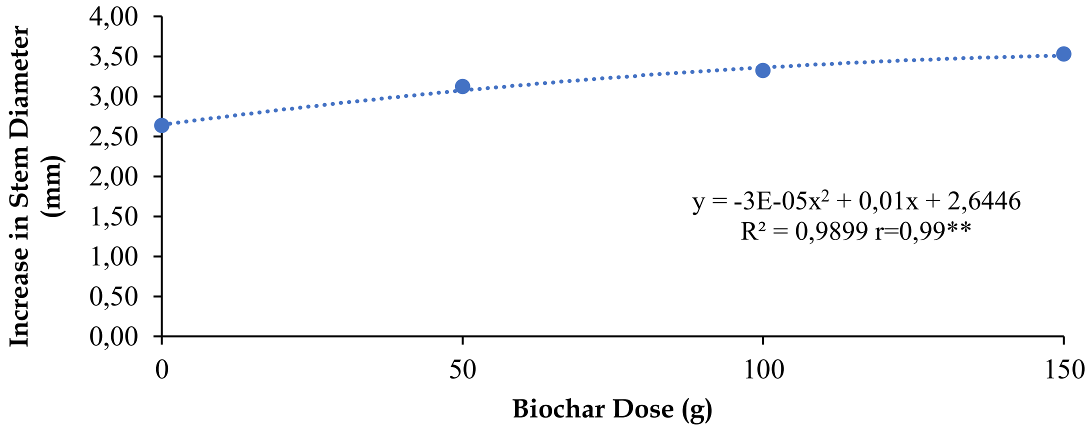

Figure 1. Bivariate Correlation Graph of Average Increase in Stem Diameter at Various Biochar Doses

The bivariate analysis in Figure 1 shows that the biochar dosage treatment responded quadratically and significantly correlated with increased stem diameter. The higher the dose of biochar given, the greater the increase in stem diameter is produced until it reaches a maximum of 5,14 at the optimum dose of 166,66 g. Furthermore, increasing the dose will result in a decrease in fresh root weight of $3E\text{-}05x^2$ for every 50 g increase, with the equation $y = -3E\text{-}05x^2 + 0{,}01x + 2{,}6446$, with diversity coefficient $R^2 = 0{,}9899$ and the correlation coefficient r = 0,99**.

3.1.2. Root fresh weight (g)

The analysis of variance showed that the biochar treatment had a significant effect. In contrast, the Moringa leaf LMO treatment and its interactions had no significant effect on the fresh root weight of sugarcane seedlings.

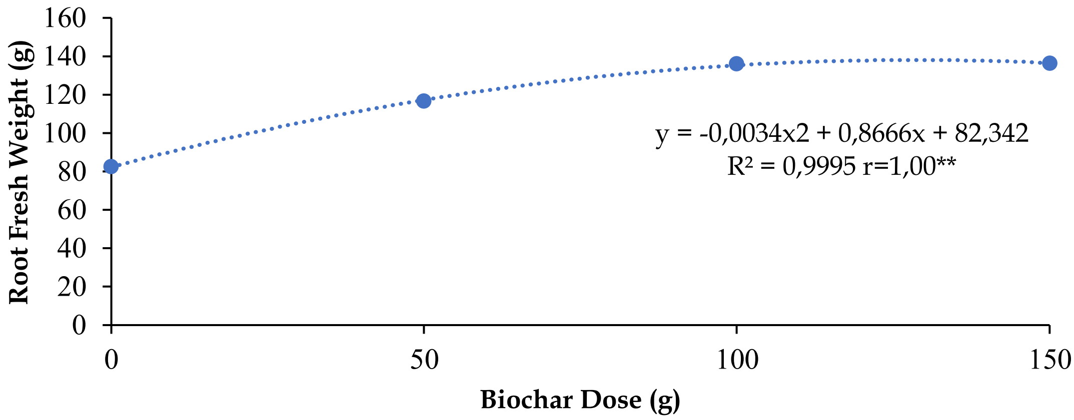

Figure 2. Bivariate Correlation Graph of Average Fresh Root Weight at Various Biochar Doses

Bivariate analysis in Figure 2 shows that the biochar dosage treatment responded quadratically and was significantly correlated with fresh root weight. The higher the biochar dose, the fresher root weight will be produced until it reaches a maximum of 248,00 at an optimum dose of 127,44 g. Furthermore, increasing the dose will result in a decrease in fresh root weight of 0,0034 for every 50 g increase with the equation $y = -0{,}0046x^2 + 1{,}0326x + 84{,}526$ with a diversity coefficient $R^2 = 0{,}9632$ and a correlation coefficient r = 1,00**.

3.1.3. *Chlorophyll a (µLMO m^{-2})*

The analysis of variance showed that the biochar treatment had a significant effect. In contrast, the Moringa leaf LMO treatment and its interactions had no significant impact on the chlorophyll content of sugarcane seedlings.

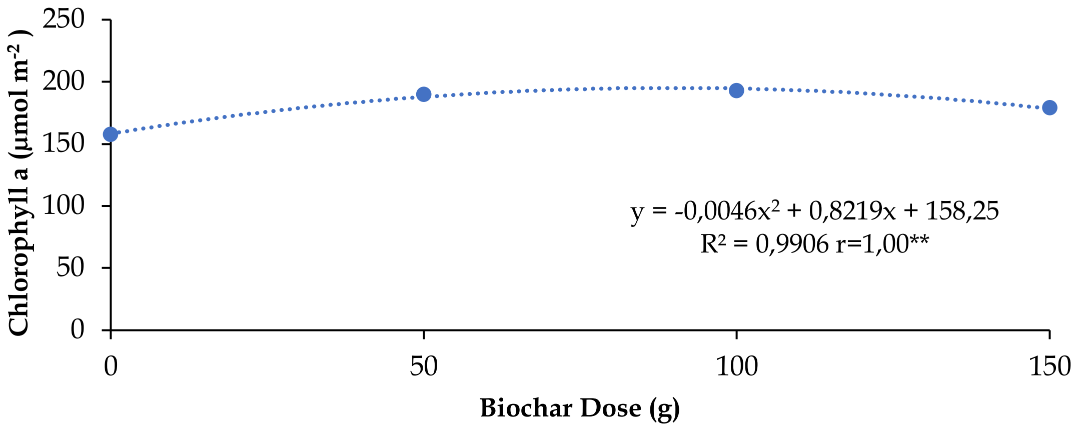

Figure 3. Bivariate Correlation Graph of Chlorophyll at Various Biochar Doses

3.1.4. *Chlorophyll b (µLMO m^{-2})*

The analysis of variance showed that the biochar treatment had a significant effect. In contrast, the LMO treatment of Moringa leaves and their interactions had no significant effect on the chlorophyll b levels of sugarcane seedlings.

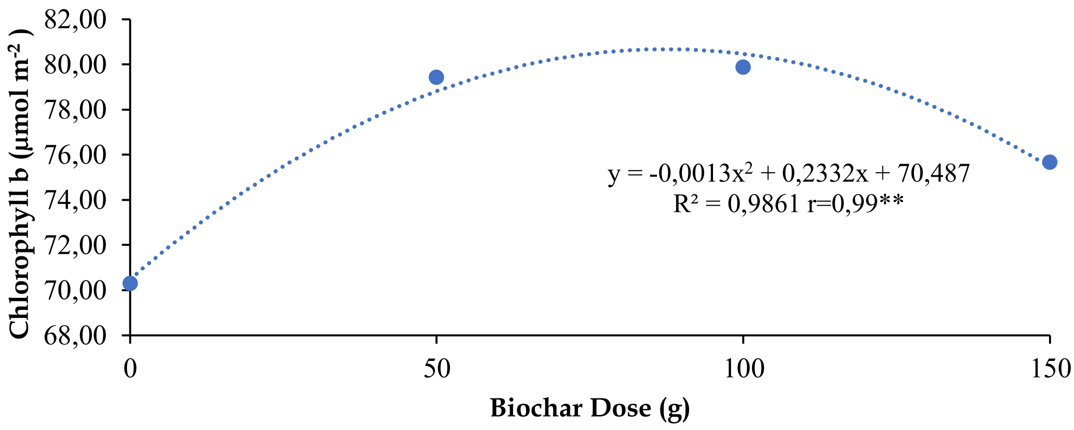

Figure 4. Bivariate Correlation Graph of Chlorophyll b at Various Biochar Doses

The bivariate analysis in Figure 4 shows that the biochar dose treatment responded quadratically and significantly correlated with chlorophyll b levels. The higher the biochar dose, the higher the chlorophyll b levels produced, reaching a maximum of 101,86 at an optimum dose of 89,69 g. Furthermore, increasing the dose will result in a decrease in fresh root weight of 0,0013 for every 50 g increase with the equation $y = -0{,}0013x^2 + 0{,}2332x + 70{,}487$ with a diversity coefficient $R^2 = 0{,}9861$ and a correlation coefficient r = 0,99**.

3.1.5. Total Chlorophyll (µLMO m^{-2})

The observations and analysis of variance in total chlorophyll levels showed that the biochar treatment had a significant effect. In contrast, the Moringa leaf LMO treatment and its interactions had no significant effect on the total chlorophyll levels of sugarcane seedlings.

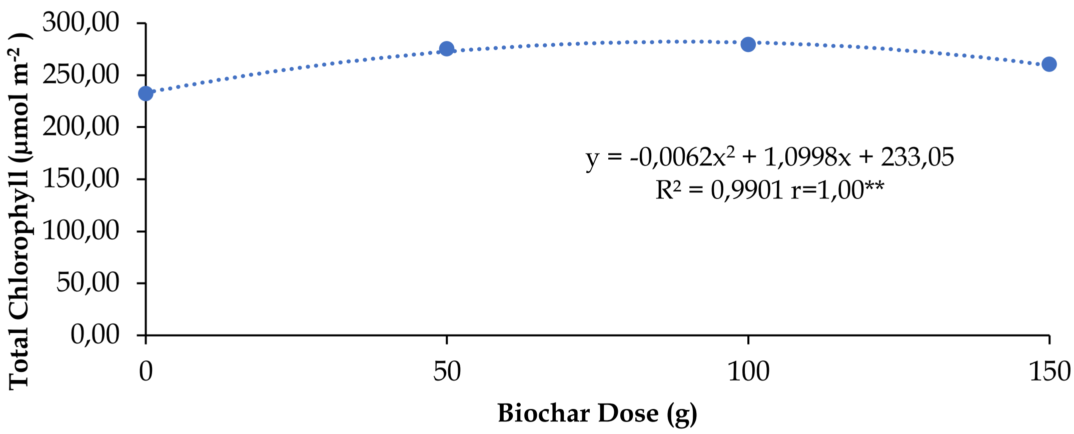

Figure 5. Bivariate Correlation Graph of Total Chlorophyll at Various Biochar Doses

The bivariate analysis in Figure 5 shows that the biochar dosage treatment responded quadratically and significantly correlated with total chlorophyll levels. The higher the dose of biochar given, the higher the total chlorophyll content produced, reaching a maximum of 379,37 at the optimum dose of 88,69 g. Furthermore, increasing the dose will result in a decrease in fresh root weight of 0,0062 for every 50 g increase with the equation y = $-0,0062x^2 + 1,0998x + 233,05$ with diversity coefficient $R^2 = 0,9901$ and the correlation coefficient r = 1,99**.

3.1.6. Number of Tillers

The analysis of variance showed that the LMO treatment of Moringa leaves had a significant effect. In contrast, the biochar treatment and its interactions had no significant effect on the number of tillers of sugarcane seedlings.

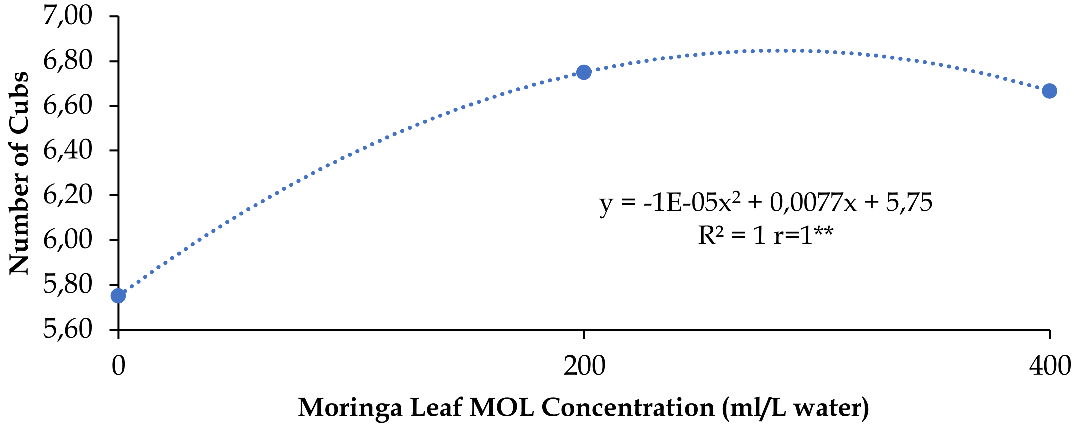

Figure 6. Bivariate Correlation Graph of Average Number of Tillers at Various Moringa Leaf LMO Concentrations

Bivariate analysis in Figure 6 shows that the LMO concentration treatment of Moringa leaves responded quadratically and significantly correlated with the number of tillers. The higher the LMO concentration of Moringa leaves, the greater the number of saplings produced, reaching a maximum of 7,23 at an optimum dose of 385 mL/L of water. Furthermore, increasing the concentration will result in a decrease in the number of offspring by $1E\text{-}05x^2$ for every 200 mL increase by following the equation y = $-1E\text{-}05x^2 + 0,0077x + 5,75$ with a coefficient of determination R2 = 1 and a correlation coefficient r = 1**.

3.2 Discussion

3.2.1. Interaction Effect of LMO Treatment of Moringa Leaves and Biochar

The research results showed no interaction between the Moringa leaf LMO treatment and sugarcane bagasse biochar on each observation variable, presumably because many factors influenced the growth of sugarcane seedlings. Internal and external factors occurred, thus causing no interaction between the Moringa leaf LMO treatment and sugarcane bagasse biochar. This follows the opinion of Tenaya et al. (2015) that the effect of interaction is the failure of one factor level relative to another factor level to provide or show the same response. The interaction effect can also be said to be the difference or difference in the response of one factor to the level of another factor, or the interaction effect is the average difference between a single influence and a simple influence.

The results of no interaction between Moringa leaf LMO and sugarcane bagasse biochar on plant growth are by the opinion of Mayrowani (2012), which states that if one factor has a more decisive influence than another factor, the other factor can be covered, and each factor has different characteristics. Different effects and nature of action, such as different types of ingredients and doses used, and different contents, will produce different relationships that influence plant growth.

3.2.2. Effect of LMO Treatment on Moringa Leaves

The results showed that the LMO treatment of Moringa leaves had different effects on each observed variable. The LMO of Moringa leaves significantly affects the number of tillers. LMO treatment of Moringa leaves with a concentration of 200 mL/L of water gave the best results on the number of tillers parameter. This indicates that using Moringa leaf LMO helps produce good vegetative growth in sugar cane plants.

The number of tillers is one of the important factors in sugarcane plants that determine the yield at harvest. The greater the number of productive tillers on the sugar cane plant, the higher the sugar cane yield will be. Giving Moringa leaf LMO to sugar cane plants produces more tillers than without giving it Moringa leaf LMO. This occurs because the LMO of Moringa leaves contains high levels of nitrogen. This is in accordance with the opinion of Suhastyo and Fanny (2021), who state that Moringa leaves contain the nutrient N and the cytokinin hormone, which plays a role in cell division and accelerates the growth of shoots and stems. Apart from that, Wu et al. (2021) stated that cytokinin influences many aspects of biological processes that influence plant growth and development, such as cell division, apical dominance, shoot initiation, and growth; Sosnowski et al. (2023), the hormone cytokinin contributes to better offspring.

From the results of the analysis of the LMO content, Moringa leaves contain 0.036% nitrogen, which helps increase plant growth. Based on the research results, applying Moringa leaf LMO to sugarcane plants significantly affects the number of sugarcane plant saplings. Laepo et al. (2018) explained that Moringa leaf LMO contains fairly balanced N and P nutrients, which are suitable for plant vegetative growth because LMO contains carbohydrates in rice washing water, glucose from brown sugar solution, and leaves. Moringa is a source of microorganisms. In addition, Zeng et al. (2020) also stated that applying N can increase the tillering rate and the number of shoots, thereby indirectly reducing the wilt heart rate. The tillering rate increases with increasing levels of N application. Sari et al. (2020) stated that Moringa leaves have good content and are needed by plants.

The microorganisms in the LMO of Moringa leaves can increase the activity of microorganisms in the soil, which is beneficial for plants. Bacteria or microorganisms in the soil can stimulate plant growth and development. This is the opinion of Lee et al. (2022), who stated that bacteria in soil affect plant growth, soil physiochemistry, and nutrient cycles. Liu et al. (2019) also noted that microbes can benefit plants in various ways, such as synthesis, release of enzymes, phytohormones, and others.

3.2.3. Effect of Biochar Treatment

The research showed that biochar treatment had different effects on each observed parameter. Biochar significantly affected the stem diameter increase, chlorophyll a, b, total levels, stem diameter, and root fresh weight. Biochar treatment with a dose of 100 g per plant gave the best results on chlorophyll A levels, chlorophyll b levels, and total chlorophyll levels. Biochar treatment with a dose of 150 g per plant gave the best results regarding the increasing stem diameter and fresh root weight.

Stem diameter is an indicator to determine whether sugarcane plants experience physiological changes. The changes are the most important factors determining whether the plant grows well. If growth is good, sugarcane sap levels can increase. According to Putri et al. (2013), the most important part of sugar production is sugar cane stalks because they contain sap, whereas in sugar cane stalks, there is thick-walled parenchyma tissue that contains liquid. Xu et al. (2021) stated that sugar cane stalks are a fresh agricultural product that contains sucrose to contribute to world sugar production. The research showed that giving biochar resulted in a suitable stem diameter, which is different from not giving biochar. This is because biochar can bind nitrogen and the nutrient content available in the soil (Nugraheni et al., 2013). Apart from that, Cunha et al. (2020) stated that nitrogen has been proven to increase stem diameter, number of internodes, and sugar productivity in sugar cane plants.

The research showed that biochar treatment significantly affected sugarcane seedlings' chlorophyll a, b, and total chlorophyll levels. Providing biochar to plants plays a positive role in leaf chlorophyll, a green pigment that is one of the main LMO in photosynthesis. In the photosynthesis process, nitrogen is needed for the formation of proteins and also chlorophyll. According to Harbinson and Yin (2022), photosynthesis drives plant productivity. Still, it is also a complex process that is highly responsive to the environment and has a complex relationship with plant growth. Plants with sufficient nitrogen content tend to have high chlorophyll content. This is also supported by the opinion of Justianti (2022), who states that the application of biochar to plants can increase the nitrogen (N) content in the soil because it has a high CEC. Khan et al., (2023) added biochar to the soil stores extra nitrogen. Preventing its loss, the carbon and wealthy functional groups on the surface of biochar can increase the soil's cation and anion exchange capacity. This is also supported by observing the greenish color of the leaves using a leaf color chart (LCC), where the average color of sugarcane seedling leaves shows a scale of 4, indicating a moderate chlorophyll level. This shows that the leaves contain sufficient chlorophyll to support the photosynthesis process.

The photosynthesis process requires water and chlorophyll to run. Biochar's ability to maintain the quality and quantity of water in the soil can help the photosynthesis process run smoothly. This is in accordance with the opinion of Karmaita et al. (2023), who state that biochar can retain water, and biochar helps plants absorb nutrients such as nitrogen, which is important for chlorophyll production, so that plants can grow and develop. The use of biochar promotes the formation of narrow and medium pores in the soil, increases soil water, increases soil water retention capacity, and optimizes water retention and soil supply capacity (Wei et al., 2033).

The research results showed that biochar treatment had a very significant effect on root fresh weight. The higher the dose of biochar given, the higher the fresh root weight produced by the plant until it reaches the optimum dose of 112.24 g. Biochar can retain water content and soil moisture. Asyifa et al. (2019) stated that biochar's ability to retain water is relatively high; its high water-holding capacity allows moisture to be maintained in the soil, creating an environmental carrying capacity. James (2004) states that sugar cane roots can penetrate the soil with a water potential of approximately -15 to -20 bar, provided the central root mass has sufficient water. Several factors influence sugarcane growth, including humidity, temperature, and available soil volume. Root growth will slow down when the soil temperature is below 18 ^{o}C and will increase progressively at an optimum temperature of 35 ^{o}C.

Moisture stress is one of the main limitations of agricultural crops, and it affects not only plant physiology but also productivity. Moisture stress can affect morphological and physiological characteristics, negatively impacting fresh weight, relative water content, and reducing nutrient diffusion. Increasing root fresh weight is important in developing tolerance to abiotic stress, especially moisture stress, because increasing root length promotes plant growth (Khan et al., 2020).

Biochar positively affects plants' growth, development, yield, and nutritional content. Jabborova et al. (2021) stated that biochar application increased root and shoot biomass. Apart from that, Joseph et al. (2021) also added that biochar availability can create favorable conditions for root development and microbial function. Biochar can catalyze biotic and abiotic reactions, especially in the rhizosphere, which increases nutrient supply and plant uptake. Ghazouani et al. (2023) stated that biochar increases micropores, which leads to an increase in the root volume ratio.

4. Conclusion

There was no interaction between Moringa leaf LMO and sugarcane bagasse biochar on all observed parameters. Moringa leaf LMO 200 mL/L water had the best effect on the number of tillers, gave a quadratic response, and significantly correlated with the number of tillers.

Biochar 100 g bagasse had the best effect on chlorophyll a, b, and total chlorophyll content. It gave a quadratic response and was significantly correlated to chlorophyll a, b, and total levels. In contrast, 150 g bagasse biochar had the best effect on increasing stem diameter and root fresh weight, and it responded quadratically and correlated very significantly with the increase in stem diameter and fresh root weight.

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