International Journal of Life Science and Agriculture Research

ISSN (Print): 2833-2091, ISSN (Online): 2833-2105

Volume 02 Issue 06 June 2023

DOI: https://doi.org/10.55677/ijlsar/V02I06Y2023-09

Page No : 137-142

Application of Rice Husk Biochar and Consortium Biological Fertilizers Against the Physiological Response of Cocoa Plants

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ABSTRACT: This study aims to determine the effect of applying rice husk biochar and consortium biofertilizers to improve the physiological processes of cocoa (*Theobroma cacao* L.) plants. This research was conducted at the Mars Cocoa Research Station Gardens in Pangkep Regency using an experimental method with the design used, namely the Two Factor Factorial (F2F) arranged in a Randomized Block Design (RBD). Four levels of rice husk biochar were used as the first factor consisting of (r0), 5kg/tree (r1), 10kg/tree (r2), and 15kg/tree (r3). The second treatment factor was the consortium biofertilizer (m) consisting of 4 levels, including without consortium biofertilizers (c0), 20 ml/L (c1), 40 ml/L (c2), and 60 ml/L (c3). Data analysis was carried out by collecting data and processing it in the form of variance (ANOVA) and then proceeding with the Tukey test with a level of alpha 0.05. The results showed that the application of rice husk biochar and consortium biofertilizers had a significant effect on the parameter Leaf Mass per Area (0,049 $g.cm^{-2}$), chlorophyll a (271,83 $mmol/m^2$), chlorophyll b (111,73 $mmol/m^2$), and total chlorophyll (389,83 $mmol/m^2$). However, the treatment did not significantly affect the application of rice husk biochar and the consortium's biofertilizers was the stomatal density parameter (264,12 mm^2).

Published Online: 23 June 2023

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KEYWORDS: Rice Husk Biochar, Cocoa (Theobroma cacao L.), Consortium Biological Fertilizer

INTRODUCTION

Cacao (*Theobroma cacao* L.) is one of the plantation crops that has been cultivated intensively by farmers in Indonesia after rubber, coffee, and oil palm. So, plant growth is one aspect that must be considered. If the needs of plants are appropriately met, then plant growth will also be good. The needs for plants to sustain life vary widely, including organic compounds and nutrients, growth regulators, the presence of microorganisms, and a good environment. If these needs are met, the physiological processes in plants can run optimally (Anggaraeni, 2020). The ongoing physiological processes are well influenced by the availability of raw materials, including nutrients and water. One of the technological developments used to promote plant physiological processes is the application of rice husk biochar and a consortium of biological fertilizers given to the planting medium.

Adding organic matter from rice husk biochar improves soil structure and fertility. According to Yulianti (2010), organic matter in the soil can improve soil aggregates, provide the nutrients needed by plants and improve the physicochemical properties of the soil. In line with Xu et. al. (2023), biochar is physicochemical and biological, so it can affect nutrient content in the soil and increase soil capacity, pH, CEC, and soil microorganism communities. The effect of biochar in the soil is closely related to the type of plant and soil fertility. The application of biochar can maintain water retention and increase the efficiency of absorption of nutrients for plants. In addition to the application of rice husk biochar, the application of consortium biofertilizers is also needed by plants because they contain several microorganisms that can support plant physiological processes. Anita (2015) stated that the quality and intensity of sunlight exposed to plants could considerably impact plant physiological processes, so sunlight can increase the work of enzyme activity as the production of metabolic substances to form chlorophyll. Based on the description above, a study was conducted on the effect of the application of rice husk biochar and consortium biofertilizers to improve the physiological processes of cocoa (*Theobroma cacao* L.) plants.

MATERIALS AND METHODS

Location and Experimental Design

The research was conducted at the Mars Cocoa Research Station in Pangkep, South Sulawesi. The research location is at coordinates 4°43’38.7” S 119°35’ 08.3” E. This research was conducted in November 2022 – April 2023. This study used an experimental method with the design used, namely the Two Factor Factorial (F2F) arranged in a Randomized Block Design (RBD). The first treatment factor is rice husk biochar (r) which consists of 4 levels, including without rice husk biochar (b0), 5kg/tree (r1), 10kg/tree (r2), and 15kg/tree (r3). The second treatment factor is consortium biofertilizer (m) consisting of 4 levels, including without consortium biofertilizer (c0), 20 ml/L (c1), 40 ml/L (c2), 60 ml/L (c3) so that there are 16 combinations of these two factors treatment. As an example, each experimental unit contained 2 MCC-02 cocoa plants with three replications, so 96 trees were used.

Making Rice Husk *Biochar*

Rice husk biochar was made by collecting rice husks around the research site. The material needed to produce rice husk biochar is 21.600 kg of rice husk, so 720 kg of rice husk biochar will be produced for the total material needed. Then the charcoal process was carried out in a pyrolysis tube for 3 hours, then the husk sorting (selection) process was carried out, which ultimately became charcoal, and then milled to obtain biochar with a homogeneous structure.

Analysis of Rice Husk B*iochar* Content

The biochar content was analyzed after the biochar was made by taking a sample of rice husk biochar and then analyzing the organic matter content at the Chemistry and Soil Fertility Laboratory, Faculty of Agriculture, Hasanuddin University. The analysis carried out included C-Organic, N-Organic content, C/N organic ratio, N, P, K, pH content, and CEC of biochar. The analysis was carried out before the research started. The rice husk biochar analysis is presented in (Table 1).

Table 1. Results of Rice Husk Biochar Analysis

Parameters	
pH	7,35
Carbon	15,74%
Nitrogen	0,92%
C/N	17
Phosphorus	0,15%
Potassium	1,87%
CEC	35,62 cmol/kg

Source: Soil Chemistry and Fertility Laboratory, Faculty of Agriculture, Hasanuddin University, 2022.

Application of Rice Husk Biochar

The application of rice husk biochar is carried out by digging in holes around the plant then the rice husk biochar is added little by little by spreading it into the planting hole according to the predetermined treatment. Furthermore, the biochar that has been applied to the planting hole is then covered with top soil from the excavation. Rice husk biochar was applied once and carried out at the beginning of the study.

Application of Concortium Biological Fertilizer

The consortium's application of biofertilizers was carried out using the ring placement method, which is to form discs around the cocoa plants at a distance of ± 75 cm from the main stem. The consortium's biological fertilizer application was carried out on cocoa plants by watering according to a predetermined treatment level by following the plate that had been formed. The consortium's biological fertilizers contain N-fixing bacteria, P dissolving bacteria, ZPT-producing bacteria, biological control bacteria, and cellulose degrading bacteria. The consortium biofertilizer was applied once per 3 months for six months with 2 liters of water per tree, and the first application was made at the start of the study (Jamil et. al., 2020).

Parameters and Data Analysis

Parameters observed in this study included Leaf Mass per Area (LMA), leaf stomatal components, leaf chlorophyll components and were observed at the end of the study after harvest. The observed parameters are calculated using the formula:

Leaf Mass per Area (LMA) = $\frac{Sample\ dry\ weight}{Sample\ leaf\ area}$ (De La Riva et al., 2016)

The stomata component of a leaf consists of 2 parts, including:

Stomatal Density = $\frac{Number\ of\ stomata}{Wide\ field\ of\ view}$ (Dama et. al., 2020)

Stomatal Opening Area = $\pi\ x\ r1\ x\ r2$ (Setiawati et. al., 2019)

Observations of the chlorophyll component are reflected in the formula that refers to (Dharmadewi, 2020), as follows:

Leaf chlorophyll content = a + b $(CCI)^c$

Keterangan:

a, b, and c = Constant

CCI = Leaf chlorophyll index data listed on *Apogee*.

Data analysis was carried out by collecting data and then tabulating it which was presented in tabular form and then processing it into the form of variance (ANOVA) and then proceeding with using the Tukey test with a level of ∝ 0.05.

RESULTS AND DISCUSSION

Table 2. Effect of Rice Husk Biochar and Consortium Biological Fertilizers on the Physiological Response of Cocoa Plants

Treatments	Parameters			
	LMA	Chlorophyll a	Chlorophyll b	Total chlorophyll
r_0c_0	0,039 b	256,23 ab	104,40 abc	367,47 ab
r_0c_1	0,042 ab	216,03 d	87,90 d	311,00 d
r_0c_2	0,049 a	260,40 ab	106,23 ab	373,43 ab
r_0c_3	0,039 b	246,70 abcd	99,87 abcd	353,80 abcd
r_1c_0	0,038 b	245,03 abcd	99,17 abcd	351,43 abcd
r_1c_1	0,039 b	271,83 a	111,73 a	389,83 a
r_1c_2	0,039 b	257,43 ab	104,80 abc	369,17 ab
r_1c_3	0,041 ab	246,60 abcd	99,83 abcd	353,67 abcd
r_2c_0	0,042 ab	252,07 abcd	102,33 abcd	361,43 abcd
r_2c_1	0,044 ab	250,20 abcd	101,47 abcd	358,90 abcd
r_2c_2	0,043 ab	258,93 ab	105,70 ab	371,37 ab
r_2c_3	0,045 ab	260,60 ab	106,17 ab	373,60 ab
r_3c_0	0,043 ab	217,17 d	88,03 d	312,40 d
r_3c_1	0,044 ab	234,67 bcd	94,83 bcd	336,87 bcd
r_3c_2	0,044 ab	219,30 cd	88,93 cd	315,37 cd
r_3c_3	0,041 ab	254,23 abc	103,30 abcd	364,57 abc
Tukey	0,0098	36,76	16,07	52,12

Note: Mean followed by the same letters are not significantly different in the Tukey test α 0,05.

Leaf Mass per Area (LMA)

The treatment of rice husk biochar and the consortium biofertilizer had significant interaction. However, singly the rice husk biochar treatment significantly affected and consortium biofertilizer treatment had no significant effect Leaf Mass per Area (LMA) (Table 2). The r_0c_2 application (without biochar husk rice + 40 ml/L consortium biofertilizer) obtained the highest average Leaf Mass per Area with a value of (0,049 $g.cm^{-2}$) which was significantly different from r_0c_0 application (without rice husk biochar + without consortium biofertilizer), r_0c_3 application (without husk rice biochar + 60 ml/L consortium biofertilizer), r_1c_0 application (5 kg/tree rice husk biochar + without consortium biofertilizer), r_1c_1 application (5 kg/tree rice husk biochar + 5 ml/L consortium biofertilizer), r_1c_2 application (5 kg/tree rice husk biochar + 40 ml/L consortium biofertilizer) and not significantly different with others treatments, while the lowest average Leaf Mass per Area was found in the application of r_1c_0 (5 kg/tree rice husk biochar + without consortium biofertilizer) which is 0,038 $g.cm^{-2}$. This is because the relative growth rate is closely related to the light efficiency of the leaves. Hence, the leaf area and net assimilation rate significantly affect the relative growth rate. In this case, the influence that can affect the rate of net assimilation is light intensity. Sunlight is one of the essential aspects in the process of photosynthesis and can be a determinant in the process of plant growth. In line with Bilman's theory (2011), the net assimilation rate is related to the level of sunlight on plants. Sunlight radiation scattered in the plant canopy is one of the determinants of the production rate of dry matter per unit leaf area during the vegetative phase.

Chlorophyll a, b, and total (μmol/m²)

The treatment of rice husk biochar and consortium biofertilizers had a significant interaction; singly, the rice husk biochar treatment also had a very significant effect, but the consortium biofertilizer treatment showed no significant effect on chlorophyll a (Table 2). Chlorophyll a, b, and total in the r_1c_1 application (5 kg/tree rice husk biochar + 20 ml/L consortium biofertilizer) obtained the highest average yield chlorophyl of cocoa plant with a value of chlorophyll a (271,83 μmol/m²), chlorophyll b (111,73 μmol/m²), and total chlorophyll (389,83 μmol/m²), which was significantly different from r_0c_1 application (without husk rice biochar + 20 ml/L consortium biofertilizer), r_3c_0 application (15 kg/tree rice husk biochar + without consortium biofertilizer), r_3c_1 application (15 kg/tree rice husk biochar + 20 ml/L consortium biofertilizer), r_3c_2 application (15 kg/tree rice husk biochar + 40 ml/L consortium biofertilizer) and not significantly different with others treatments, while the lowest average chlorophyll in cocoa plants was in the application r_0c_1 (without biochar rice husk + 20 ml/L consortium biofertilizer) is chlorophyll a (216,03 μmol/m²), chlorophyll b (87,90 μmol/m²), and total chlorophyll (311,00 μmol/m²). The application of rice husk biochar and the consortium's biological fertilizers in balanced doses recorded significant results in measuring chlorophyll content because the application of rice husk biochar could bind water, while the application of the consortium's biological fertilizers assisted in providing nutrients. In this case, water is essential in dissolving soil nutrients so plants can utilize them. The availability of dissolved nutrients is needed for plant physiological processes, one of which is the formation of chlorophyll in photosynthesis (Lakitan, 2013). Dharmadewi (2020) stated that chlorophyll a and b have a role in food photosynthesis. Chlorophyll b is a photosynthetic antenna for plants to collect light and divert it to the reaction center. Meanwhile, the composition of the reaction center comes from chlorophyll a. In addition, according to Firmansyah (2019), the higher the rate of photosynthesis in the photosynthesis process, the higher the amount of chlorophyll obtained, followed by the more assimilates formed. The assimilate formed will then be translocated to parts of the plant that need it, for example fruit and help the process of forming and filling seeds so that plant growth will be better and production will increase.

Stomatal Density (mm²)

Treatment of rice husk biochar and biofertilizer consortium with various concentrations and their interactions did not significantly affect the stomata density of cocoa plants (Figure 1). The Stomata density of cocoa in the application r_2c_1 (10 kg/tree rice husk biochar + 20 ml/L consortium biofertilizer) obtained the highest average stomata density of cocoa plants with a value of 264,12 mm². In contrast, cocoa pods' lowest average stomata density was in the r_0c_3 treatment (without rice husk biochar + 60 ml/L consortium biofertilizer) with a value of 227,60 mm². Stomata density is the number of stomata on a leaf. However, according to the results obtained, the application of rice husk biochar and consortium biofertilizers did not affect the stomatal density parameter. This is due to similar species and the same planting location as well as physiological aspects, which include the intensity of sunlight and rainfall, causing the density of stomata on each plant and the relative treatment to obtain the same results or not to show significant differences. According to Fitter & Hay (1991), physiologically, light has effects directly or indirectly. The direct effect of light can be seen in photosynthesis, whereas the effect of light indirectly can be seen in the growth and development of plants caused by the direct response of metabolites. Rodriguez et al. (2017), stomata density can have a significant effect due to plant genetics and the surrounding environment, as it is known that groups of plants that receive high light produce smaller and thicker leaf sizes, as well as a smaller number of stomata, meanwhile, plants that receive low light results in wider leaves and wider spaces between cells so that the number of stomata will also be greater.

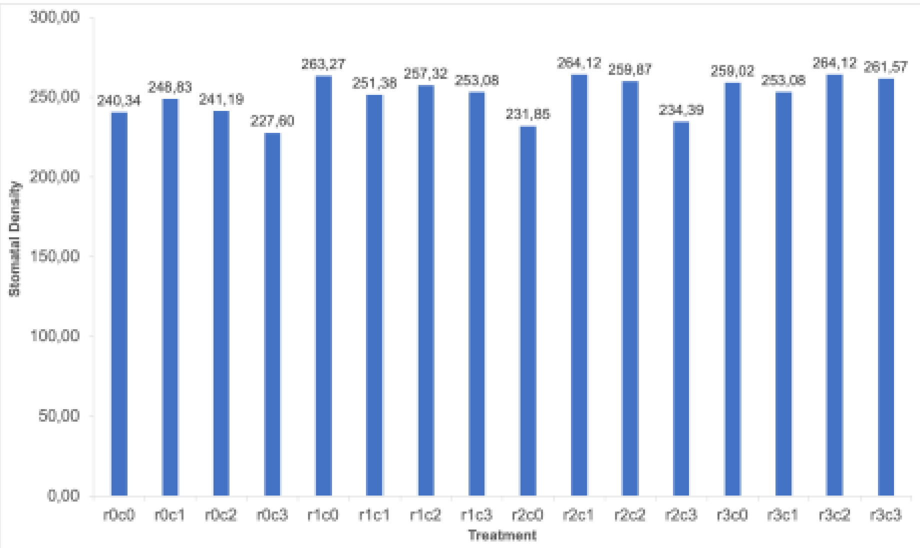

Figure 1. Bar Chart Density of Stomata in Cocoa Plants Against Biochar Application of Rice Husk and Consortium Biofertilizers

Stomatal Opening Area (μm^2)

There was no interaction between the treatment of rice husk biochar and the consortium's biological fertilizers. However, the rice husk biochar treatment had a significant effect, but the consortium's biofertilizer treatment did not significantly affect the area of stomatal openings (Table 3). The area of stomatal openings of cocoa plants in applications r_1 (5 kg/tree rice husk biochar) obtained the highest average yield with a value of (41,38 μm^2), which was significantly different from b_0 (without rice husk biochar), but not significantly different from b_2 (10 kg/tree rice husk biochar) and b_3 (15 kg/tree rice husk biochar). It is known that potassium is a nutrient that has an essential role in the process of opening and closing stomata. According to Jasmi (2016), the opening of the stomata will occur when the pressure on the guard cells increases. Turgor pressure on the guard cells increases due to water entering the guard cells. The movement of water entering the guard cells from one cell to another always starts with the cell with the higher water potential and then moves on to the cell with the lower water potential. Factors that cause stomata to open and close include turgor mechanisms and environmental factors, including temperature, light, and humidity. Guard cells are stimulated by sunlight to absorb K^+ ions and water so that the stomata open in the morning. In plants, stomata have a vital role as a tool used to adapt so that plants avoid drought stress. In this case, potassium is closely related to osmotic potential, if the plant lacks water, potassium can reduce the osmotic potential so that it will cause stomata to close. Therefore, water shortages caused by the transpiration process can be minimized. According to Hardjowigeno (2007), the application of potassium can affect yield, not only playing a role in the process of photosynthesis and respiration but also in the process of forming starch, as an activator of enzymes, the process of opening and closing stomata, and other physiological processes in plants. In addition, potassium also plays a role in increasing plant resistance to drought and disease attacks.

Table 3. Average Stomatal Opening Area in Rice Husk Biochar Application and Consortium Biological Fertilizers

Rice Husk Biochar (kg/tree)	Fertilizer Biological Consortium (ml/L)				Average
	0 (c0)	20 (c1)	40 (c2)	60 (c3)	
0 (r0)	19,23	27,31	28,79	22,83	24,54 b
5 (r1)	41,47	40,53	38,92	44,58	41,38 a
10 (r2)	39,45	35,98	34,28	38,14	36,96 a
15 (r3)	33,56	32,35	41,61	38,20	36,43 a
Tukey	5,46				

Note: Means followed by the same letter are not significantly different in the Tukey test α 0,05.

CONCLUSIONS

It can be concluded that the application of rice husk biochar and consortium biofertilizers had a significant effect on the parameter Leaf Mass per Area (0,049 g.cm^{-2}), chlorophyll a (271,83 mmol/m^2), chlorophyll b (111,73 mmol/m^2), and total chlorophyll (389,83 mmol/m^2). Meanwhile, the single application of rice husk biochar obtained significant results on the stomata opening area parameter (41,38 μm^2). However, the treatment did not significantly affect the application of rice husk biochar and the consortium's biofertilizers was the stomatal density parameter (264,12 mm^2). Lastly, applying rice husk biochar and consortium biofertilizers gave cocoa plants an excellent physiological response.

ACKNOWLEDGEMENTS

The researcher proposes to thank the *Mars Cocoa Research Station*, Pangkep Regency, South Sulawesi, which has facilitated this research so that it can be carried out. The same remarks were conveyed to Mr. Agus Purwantara, Ph. D (Site Manager of Mars Cocoa Research Station) and brother Achmad Ferdiansyah (Mars Cocoa Research Station staff) who helped during the research.

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