

Application of Coconut Shell Biochar and Rhizobacteria Consortium to Increase Cocoa Production

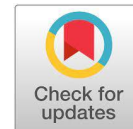
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Abstract

The decline in Indonesia's cocoa production as a result of soil fertility degradation causes fertilization to become one of effective efforts. Because of that, it is possible to restore soil fertility potential by using soil fertility enhancer biochar combined with rhizobacteria consortium to create suitable conditions for plant growth. This research was aimed to study and investigate coconut shell biochar interacts with rhizobacteria in increasing cocoa production. This research was conducted at Kodeoha, North Kolaka, Southeast Sulawesi, Indonesia, from September 2022 until March 2023. A split plot design with three replications each consisted of two experimental units was used as an experimental design. Biochar dosage as the main plot consisted of four levels, namely 0 ton ha⁻¹ (control), 2.5 tons ha⁻¹, 5 tons ha⁻¹, and 7.5 tons ha⁻¹ and rhizobacteria concentration as the sub plots consisted of four levels, namely 0 mL L⁻¹, 50 mL L⁻¹, 100 mL L⁻¹, and 150 mL L⁻¹. Results of this research showed a significant interaction between biochar 5 tons ha⁻¹ and rhizobacteria 100 mL L⁻¹ which provide best result for chlorophyll a (336 μmol m⁻²), chlorophyll b (150 μmol m⁻²), and total chlorophyll (484 μmol m⁻²). The interaction between biochar 5 tons ha⁻¹ and rhizobacteria 150 mL L⁻¹ showed the best results for number of harvested pods per tree (10.00 pods), bean weight per tree (606 g trees⁻¹), stomata opening area (31 μm²). The interaction between biochar 7.5 tons ha⁻¹ and rhizobacteria 150 mL L⁻¹ showed the best results on the weight of 100 dry beans at water content 8% (159.03 g).

Keywords: Biochar, cocoa, production, rhizobacteria

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is classified as an industrial plantation crop which is in Indonesia managed by large state and private companies, but most managed by smallholders. Indonesia continuously develop cocoa plantations in many provinces. According to data of International Cocoa Organization (2021), which shows that Indonesia has produced 3.2% of cocoa globally and is the world's seventh largest producer after Ivory Coast, Ghana, Ecuador, Cameroon, Nigeria, and Brazil.

Indonesia's cocoa production in recent years has tended to decrease every year including in North Kolaka (BPS, 2022). The decline in Indonesia's cocoa production lately is considered due to the problem of soil fertility degradation in cocoa planting areas. This condition is caused by erosion, nutrient leaching, inappropriate management practices, and nutrient exploitation after harvest. In every 1,000 kg of cocoa beans harvested, 546 kg of N, 96 kg of P, 246 kg of K, and other nutrients such as Mg, Ca, and Fe are transported (Sobari *et al.*, 2014).

Responding to the many problems that occur, an alternative soil amendment is needed. One alternative of the soil fertility enhancer that can be used is biochar. Biochar is a natural material that is formed through the process of burning biomass in limited or no oxygen conditions so that it can be utilized by the soil as a source of organic matter (Nurida *et al.*, 2012).

One of the raw materials that can be used to make biochar is coconut shell. The potential for coconut shells is enormous considering that the total area of coconut plantations in Indonesia reaches 3.36 million hectares with a production of 2.78 million tons year⁻¹, where the proportion of the shell can reach around 15-19% (BPS, 2021).

It has been demonstrated that adding biochar made from coconut shells may raise pH and CEC of soil. Leaching of cations like K⁺ and NH₄⁺ will be reduced by adding charcoal to increase soil CEC. The increased crop yield is undoubtedly impacted by the improvement in soil quality. This is supported by Nurida *et al.* (2012), who found that adding 5 tons ha⁻¹ of coconut shells biochar increased maize yields in terms of dry cob weight (2.37 tons ha⁻¹), dry biomass weight (4.04 tons ha⁻¹), and dry shelled weight (1.85 tons ha⁻¹).

The biochar application cannot replace the role of fertilizer, therefore it is necessary to add substances that make soil' as well as fertilizer' nutrients more available for plants. Rhizobacteria are an alternative treatment which can applied on around plant roots by synthesizing and regulating the concentration of various growth regulators (Husen *et al.*, 2006).

Rhizobacteria utilization has been prevalent in a variety of horticulture crops, from food to annual or plantation crops. According to the findings of Irawan *et al.* (2022), cocoa seedlings' height, number of leaves, stem diameter, fresh and dried weight of roots,

number of roots, and root volume could all be increased by application of 100 mL L⁻¹ cocoa root rhizobacteria. According to the findings of a different study by Nasaruddin *et al.* (2019), applying rhizobacteria three times enhanced leaf area, leaf moisture content, chlorophyll a, b, and total chlorophyll in cocoa leaves.

The interaction between the application of biochar and rhizobacteria can be seen from the ability of biochar to provide a good and suitable habitat for soil microbes by improving the physical condition of the soil. By creating good soil and environmental conditions, it is certain that the process of degradation by microbes will increase (Gumelar & Yunus, 2021).

Based on the explained background above, the problem may be stated as how coconut shells biochar interacts with rhizobacteria in increasing cocoa production. This research purpose was to study and investigate interaction effects of the application of coconut shell biochar and rhizobacteria on growth and production of cocoa.

MATERIALS AND METHODS

The research was conducted at a small-holder cocoa plantation in Kodeoha, North Kolaka, Southeast Sulawesi, Indonesia with alluvial soil type and an average rainfall is 91.5 mm per month during 6 months period of this study. The coordinate of the study site is between 3°20'0"S-3°30'0"S and extends from west to east between 120°55'0"E-121°10'0"E. The trial was carried out from September 2022 until March 2023. The short duration of observation (6 months) might be a limitation of this study. Cocoa is a perennial crop where their growth is affected by various long-term factors. Hence, the result of this study shouldn't be generalized. Further observation over a longer duration should be done to further investigate the effect of the treatment.

The research used three months old MCC 02 cocoa clones under the shade of coconut trees; derived from shoot grafting which was planted at a spacing of 3 m x 3 m. The research was carried out as an experiment using a split plot design with the main plot consists of 4 levels of coconut shell biochar, 0 tons ha⁻¹ (control), 2.5 tons ha⁻¹, 5 tons ha⁻¹, and 7.5 tons ha⁻¹. The subplots consisted of 4 levels concentrations of the Rhizobacteria solution, 0 mL L⁻¹ (control), 50 mL L⁻¹, 100 mL L⁻¹, and 150 mL L⁻¹. The density of Rhizobacteria was stated in Table 1. Each treatment was repeated three times with two sample plants for each experimental unit so that there were 96 experimental units.

The following is a description of the study's implementation strategy. First, before treatment begins, pruning is done with the intention of removing branches that are dry and infested by pests and diseases. Additionally, weeding is done by clearing the area around the crown circle. Second, preparation of biochar started with material of coconut shells wastes collected from farmer surrounding the research site. The coconut shell were dried under the sun for 7 days. A basic vertical pyrolysator composed of 2 drums was used with combustion temperature between 300 °C and 560 °C. After 3.5 hours of combustion, the biochar was immediately sprayed with water to keep it from turning into ashes, then dried in the sun and crushed until the particle diameter was less than 2 mm.

Third, preparation of Rhizobacteria consortium which contains bacteria as Table 1. One plant receives 1,000 mL of the Rhizobacteria consortium solution and 12 plants are used

in each concentration with two experimental units.

Fourth, the process of inoculation involved combining coconut shell biochar (the carrier) with Rhizobacteria as biodecomposers for each treatment and then letting it rest for about 14 days before it was ready to be applied to the soil.

Fifth, application treatment by making arrays around the cocoa plant in accordance with predefined treatments levels. The treatment was applied once at the beginning of the study in accordance with the prepared disks. Around the plantations, the treatment that has been given to the plants is then covered with litter.

Sixth, application of basic fertilizer in the form of 16:16:16 NPK fertilizer was carried out once at the beginning of the study by making arrays around the cocoa plant disks at a distance of 1–1.5 m from the cocoa plant stems and then covered with litter.

Seventh, the harvesting of cocoa pods begins in January until March 2023. The requirements for harvesting MCC 02 pods were that immature pods were red at first and became dark orange when ripe.

Observations were made on parameters of number of harvested pods, weight of 100 dry beans which was observed after drying at water content 8%, production per trees by counting all mature pods per trees every time when the pods is ripe, chlorophyll a, chlorophyll b, total chlorophyll, and stomatal opening area at the end of the study.

Table 1. Composition and bacterial density of Rhizobacteria consortium

Rhizobacteria composition	Bacterial density
<i>Azotobacter</i> sp.	2.25 x 10 ⁴ CFU mL ⁻¹
<i>Pseudomonas</i> sp.	2.25 x 10 ⁷ CFU mL ⁻¹
<i>Lactobacillus</i> sp.	5.47 x 10 ⁷ CFU mL ⁻¹
<i>Rhizobium</i> sp.	4.25 x 10 ⁸ CFU mL ⁻¹
<i>Streptomyces</i> sp.	3.25 x 10 ⁷ CFU mL ⁻¹
Nitrogen fixing bacteria	4.67 x 10 ⁸ CFU mL ⁻¹
Phosphate solubilizing bacteria	2.54 x 10 ⁴ CFU mL ⁻¹

Data was analyzed by analysis of variance (ANOVA). If there were a significant effect, it will be continued with further tests using the Least Significant Difference (LSD) $\alpha = 0.05$ and orthogonal polynomial tests.

RESULTS AND DISCUSSION

Based on the ANOVA, it was found that there was a significant interaction between biochar and Rhizobacteria treatments on the number of harvested pods, weight of 100 dry beans, production per tree, chlorophyll a, chlorophyll b, total chlorophyll, and stomatal opening area.

The results of the formation of harvested pods (Table 2) show that the ability of Rhizobacteria to optimize pods development also indicates a strong interaction in the rhizosphere. When the biochar contacts with root hairs, there will be a potential difference between the roots and the soil so the Rhizobacteria will be induced to increase their capacity to release nutrient ions and electrons. This is in accordance with the opinion of Yu & Kuzyakov (2021), that a difference in the potential of the root membrane will cause biochar to act like a circuit board between resistors and capacitors which can release and even store nutrient ions and electrons when needed by plants at any time.

Other studies have assessed the effect of application of PGPR (plant growth promoting Rhizobacteria) and biochar on soil quality

defining physicochemical and biological properties of soils. For instance, co-application of biochar with PGPR has generally been found to increase the mineral nutrient content in soils when compared to sole application of either biochar or PGPR. Ren *et al.* (2020) found that using *Bacillus megaterium* with wheat-derived biochar increased available nitrogen, phosphorus, and total potassium in PGPR+biochar treatment by 68%, 45%, and 21%, respectively, than PGPR only and by 22%, 16%, and 30%, respectively than biochar only treatment. Jabbarova *et al.* (2020) evaluated that shoot nitrogen was 1.64 mg N g⁻¹ shoot in soil treated with PGPR (*Bacillus* sp.) co-inoculated with biochar, which was significantly higher than that in sole applications of *Bacillus* sp or biochar.

Overall these studies indicated that co-application of biochar and PGPR works in synergy to raise the nutrient level higher than the individual application of either biochar or PGPR. Biochars contain macro as well as micro-nutrients. When applied to soils, they contribute nutrients to soils as a result of dissolution and decomposition under the influence of soil conditions and microbial activity. The PGPR particularly those solubilizing the organic phosphate, apparently accelerates the available phosphorus from biochar. Soil microorganisms, PGPR and biochar facilitate the availability of nutrients and leading to enhancing enzymatic activity (Zheng *et al.*, 2022).

In relation to the development of cocoa pods, another important aspect that supports

Table 2. Effect of interaction of biochar and Rhizobacteria on the number of harvested pods per tree during the study period

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r ₀ (control)	r ₁ (50 mL L ⁻¹)	r ₂ (100 mL L ⁻¹)	r ₃ (150 mL L ⁻¹)	
Harvested pods per tree					
b ₀ (control)	3.50 a P	3.50 b P	4.67 b P	3.83 c P	2.00
b ₁ (2.5 tons ha ⁻¹)	4.50 a P	6.17 a P	5.67 b P	6.17 b P	
b ₂ (5 tons ha ⁻¹)	4.00 a R	6.50 a Q	8.00 a Q	10.00 a P	
b ₃ (7.5 tons ha ⁻¹)	4.83 a Q	7.50 a P	8.00 a P	8.00 b P	
LSD (r)	2.06				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test $\alpha = 0.05$.

plant productivity is the dry weight of the beans. Dry bean weight is a reflection of energy by plants during the process of photosynthesis. From Table 3 it can be seen that the increase of dry beans weight corresponds to an increase in the dose of biochar and the concentration of rhizobacteria. This is due to the interaction between the two treatments which supports the development of microbes in the soil so that the regulation of sucrose phosphate synthase (SPS) and sucrose synthase (SS) to catalyze the cleavage of sucrose into UDP-glucose and fructose becomes higher. This is in accordance with the opinion of Wang *et al.* (2013), who stated that increased cellulose synthesis and secondary wall deposition consistently correlated with increased SPS activity because SPS would allow resynthesizing sucrose substrate for cell wall biosynthesis. The above opinion was also reinforced by Osorio *et al.* (2014), which stated that a sufficiently large allocation of carbon in plant organs caused the expression of the SPS gene to increase and had an impact on the amount of sucrose in the pods much higher so that the total number of pods and total weight pods increases.

The interaction that occurs in the research results (Table 4) is due to the synergy of biochar in providing suitable habitat for the development of Rhizobacteria. These bacteria can carry out their metabolic activities properly due to the sufficient energy needed in the form of C-organic from the biochar. This can be seen in the results of soil analysis after treatment (Table 10) that there was an increase in soil organic C as the dose of biochar was increased. Generally, rhizobacteria also synergize with organic matter and other factors in the soil, but the high organic C content produced by biochar itself supports the synergy of rhizobacteria. It has also been proven that a high organic C content in the soil is able to increase the population of soil microbes thereby indirectly increasing the

efficiency of nutrient absorption to be utilized by plants. Providing a relatively high source of organic matter through rhizobacteria can increase the efficiency of soil microbes to increase soil fertility which has an impact on increasing plant growth and production. This is in line with a similar study conducted by Antonius *et al.* (2018), which explained that the number of soil microorganisms in the field is strongly influenced by the balance of organic matter and good soil fertility conditions.

The high yield potential shown there was an increase in production per hectare by 73.53% is inseparable from the role of Rhizobacteria. There is support for the relatively small size of the biochar particles, which allows the ability to be degraded by bacteria to increase. The smaller surface morphological structure of the biochar, the micro pores with the utilization of functional groups will provide opportunities for positive or negative charges from the surface of the biochar during hydrolysis in the soil. The positive or negative charge that arises can increase the exchange of cation or anion soil nutrients for further use by plants. This statement is in accordance with the opinion of Abel *et al.* (2013), which states that the particle size of biochar greatly influences the distribution of soil pore sizes.

The results obtained also showed that the lowest production was in the control treatment. This is due to the degradation of soil physical properties as a result of cropping conditions which usually flood and have an impact on inhibiting pods development. The condition without soil amendments occurs due to a lack of oxygen supply to the roots and high levels of salinity (Table 10). This statement is in accordance with the opinion of Sari *et al.* (2021), which states that flooding causes disruption of aeration in the soil. Excessive addition of water can cause the volume of air pores in the soil to be filled with water, so the diffusion of oxygen in the root

area is obstructed. If there is excess oxygen in the root area, it will result in a change from aerobic respiration to anaerobic respiration and in the end the energy (ATP) produced will be lower. If energy is limited, the uptake and translocation of water and nutrients for plant growth will be obstructed.

Increasing the photosynthetic activity of plants will depend on the availability and uptake of sufficient nutrients. Stomatal opening area is one sign that is closely connected to photosynthesis. Based on results (Table 5) showed that 5 ton ha⁻¹ coconut shell biochar produced the largest average stomatal opening area. This is due to the coconut shell biochar's ability to grow roots that are strong enough

to hold and retain water in the proper proportion, as well as the existence of mineral fraction content, so that the optimization of photosynthesis can be achieved. As a result of photosynthesis, the guard cells that protect stomata cause a gradient (difference) in their osmotic value which allows water to enter. Turgidity also occurs, causing the cell walls to expand and the stomata open wider. This statement is supported by Felania (2017), which states that increasing the capacity of roots to absorb water and nutrients would impact the size of stomata openings as a result of increasing the cell turgor in leaves.

An increase in turgor in the cell wall causes an increase in pods production. This

Table 3. Interaction of biochar and Rhizobacteria on the weight of 100 dry beans (g) at water content 8%

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r ₀ (control)	r ₁ (50 mL L ⁻¹)	r ₂ (100 mL L ⁻¹)	r ₃ (150 mL L ⁻¹)	
Weight of 100 dry beans (g)					
b ₀ (control)	137.7 a PQ	143.7 b P	127.3 c Q	133.7 b PQ	1.97
b ₁ (2.5 tons ha ⁻¹)	14.4 a P	150.5 ab P	152.1 ab P	143.2 b P	
b ₂ (5 tons ha ⁻¹)	139.8 a Q	158.8 a P	142.1 b Q	153.9 ab PQ	
b ₃ (7.5 tons ha ⁻¹)	121.11 b Q	111.3 c Q	154.3 a P	159.0 a P	
LSD (r)	12.92				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test α 0.05.

Table 4. Effect of interaction of biochar and Rhizobacteria on the bean yield per tree (g)

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r ₀ (control)	r ₁ (50 mL L ⁻¹)	r ₂ (100 mL L ⁻¹)	r ₃ (150 mL L ⁻¹)	
Bean yield per tree (g)					
b ₀ (control)	162.8 a P	159.2 b P	190.6 b P	184.8 c P	132.40
b ₁ (2.5 tons ha ⁻¹)	220.0 a Q	319.3 a P	295.4 b P	319.6 b P	
b ₂ (5 tons ha ⁻¹)	187.7 a R	348.8 a Q	448.9 a Q	605.6 a P	
b ₃ (7.5 tons ha ⁻¹)	191.1 a R	329.3 a Q	447.6 a PQ	530.4 a P	
LSD (r)	142.34				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test α 0.05.

Table 5. Effect of interaction of biochar and Rhizobacteria on the stomatal opening area (μm^2)

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r ₀ (control)	r ₁ (50 mL L ⁻¹)	r ₂ (100 mL L ⁻¹)	r ₃ (150 mL L ⁻¹)	
Stomatal opening area (μm^2)					
b ₀ (control)	15.44 a Q	14.92 c Q	21.20 a PQ	24.60 b P	5.39
b ₁ (2.5 tons ha ⁻¹)	15.44 a R	21.20 b Q	24.60 a PQ	28.26 ab P	
b ₂ (5 tons ha ⁻¹)	17.01 a R	29.05 a P	23.03 ac Q	30.88 a P	
b ₃ (7.5 tons ha ⁻¹)	19.36 a Q	18.32 bc Q	24.34 a PQ	28.00 ab P	
LSD (r)	5.43				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test α 0.05.

is due to the accumulation of sucrose which was initially stored in vacuoles in the parenchymal cells of the stem's vacuoles and then induced to release sugar to help fill the beans. Parenchyma cells in cocoa stems and branches collect sugar to a level that has a greater osmotic potential value when biochar is present. This was in agreement with Wang *et al.* (2013) who stated that, as part of the dynamic equilibrium between turgor-induced absorption and leakage before, an increase in turgor pressure can encourage cells to adjust by releasing sugar back to the apoplast.

In addition to the stomatal components, the study's findings also include other physiological components, such as chlorophyll to support the idea that coconut shell biochar combined with Rhizobacteria can produce all the elements required for plants to grow and develop effectively. The results (Table 7–9) showed that 5 ton ha⁻¹ biochar with 100 mL L⁻¹ Rhizobacteria had a significant difference with the control treatment. The ability of the plant to absorb the outcomes of transferring

sunlight to the leaf surface greatly influences the variation in the amount of chlorophyll that is generated in the leaves. The two treatments above produced the best conditions, which improved the efficiency that the sunlight was absorbed by the leaves, which also affected the leaf thickness. Thicker leaves are able to absorb and assimilate more nutrients, so the chlorophyll content also increases. Dharmadewi (2020), who claims that the thickness of the leaf tissue could influence the chlorophyll content, supports this claim by mentioning that thin leaves often wilt rapidly, which degrades the chlorophyll as well.

The increase in chlorophyll content formed is also the result of an increase in nutrient absorption, including element K. This is supported by the results of the soil analysis (Table 10) which shows a positive increase between total K and chlorophyll. This is caused by increased mineralization and utilization of potassium through enzyme activity as a consequence of amendments to biochar which have a high soil retention capacity.

Table 6. Effect of interaction of biochar and Rhizobacteria on the chlorophyll a (µmol m⁻²)

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r ₀ (control)	r ₁ (50 mL L ⁻¹)	r ₂ (100 mL L ⁻¹)	r ₃ (150 mL L ⁻¹)	
Chlorophyll a (µmol m ⁻²)					
b ₀ (control)	214 b Q	231 b Q	281 a P	270 a PQ	46
b ₁ (2.5 tons ha ⁻¹)	260 ab P	261 b P	256 b P	298 a P	
b ₂ (5 tons ha ⁻¹)	247 ab Q	308 a PQ	336 a P	276 a Q	
b ₃ (7.5 tons ha ⁻¹)	277 a P	260 b P	273 a P	291 a P	
LSD (r)	47				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test α 0.05.

Table 7. Effect of Interaction of biochar and Rhizobacteria on the chlorophyll b (µmol m⁻²)

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r ₀ (control)	r ₁ (50 mL L ⁻¹)	r ₂ (100 mL L ⁻¹)	r ₃ (150 mL L ⁻¹)	
Chlorophyll b (µmol m ⁻²)					
b ₀ (control)	87 b Q	95 b PQ	116 b P	111 a P	23
b ₁ (2.5 tons ha ⁻¹)	106 ab P	108 b P	105 b P	126 a P	
b ₂ (5 tons ha ⁻¹)	101 ab Q	132 a PQ	150 a P	114 a Q	
b ₃ (7.5 tons ha ⁻¹)	114 a P	106 b P	113 b P	122 a P	
LSD (r)	24				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test α 0.05.

Table 8. Effect of interaction of biochar and Rhizobacteria on the total chlorophyll ($\mu\text{mol m}^{-2}$)

Dosage of biochar	Concentration of Rhizobacteria				LSD (b)
	r_0 (control)	r_1 (50 mL L ⁻¹)	r_2 (100 mL L ⁻¹)	r_3 (150 mL L ⁻¹)	
	Total chlorophyll ($\mu\text{mol m}^{-2}$)				
b_0 (control)	308 b Q	333 b Q	403 b P	388 a PQ	65
b_1 (2.5 tons ha ⁻¹)	372 ab P	375 b P	367 b P	428 a P	
b_2 (5 tons ha ⁻¹)	352 ab Q	442 a PQ	484 a P	396 a Q	
b_3 (7.5 tons ha ⁻¹)	397 a P	372 b P	391 b P	418 a P	
LSD (r)	67				

Notes: Numbers followed by the identical letters in the same columns (a,b,c) or the same rows (P,Q,R) are not statistically different in the LSD test α 0.05.

Table 9. Effect of Rhizobacteria application on several soil chemical characteristics

Rhizobacteria treatment (mL L ⁻¹)	Walkley & Black C (%)	Kjeldahl N (%)	C/N	CEC (meq%)	P (%)	K (%)
50	23.9	1.49	16	40.2	0.36	1.51
100	24.8	1.07	23	52.9	0.49	1.08
150	21.4	1.21	18	39.1	0.45	0.64

Source: LKKT, 2022.

Table 10. Effect of biochar and Rhizobacteria combination application on several soil chemical characteristics

Treatment	pH	Walkley & Black C (%)	Kjeldahl N (%)	C/N	Olsen P ₂ O ₅ (ppm)	K (me%)	CEC (me%)
Before	6.33S1	1.93S2	0.19S2	10	10.9S2	0.52S1	11.4S2
After (B0R0)	6.24S1	1.59S2	0.21S1	8	12.5S2	0.50S1	12.2S2
After (B1R3)	6.57S1	1.95S2	0.21S1	9	17.5S1	0.56S1	12.9S2
After (B2R2)	6.63S1	2.09S1	0.15S1	14	16.6S1	0.56S1	13.0S2
After (B3R3)	6.44S1	2.13S1	0.18S2	12	17.1S1	0.53S1	12.4S2

Notes: B0R0: Control, B1R3: Biochar 2.5 tons ha⁻¹ with Rhizobacteria 150 mL L⁻¹, B2R2: Biochar 5 tons ha⁻¹ with Rhizobacteria 100 mL L⁻¹, and B3R3: Biochar 7.5 tons ha⁻¹ with Rhizobacteria 150 mL L⁻¹; Criteria for Land Suitability for Cocoa (Puslitkoka, 2004); S1: Highly suitable and S2: Fairly suitable; Source: LKKT, 2022 and 2023.

The above statement is supported by Ardiansyah *et al.* (2022), who explained that the availability of K content as one of the elements that forms chlorophyll can provide a stimulant for increasing total chlorophyll. This condition is also supported by the research of Suharja & Sutarno (2009), which shows that there is a significant effect of fertilization on chlorophyll content indicating that the supply of nutrients (N, P, K, Mg, S) has a positive contribution to the formation of chlorophyll in the leaves of Sakti chili plants.

Improved soil conditions can be seen in the results of soil analysis after treatment (Table 10). The increase in soil chemical components during the study may increase in microbial enzymatic performance in the soil, such as nodulation process. A research

by Vanek *et al.* (2016) showed that biochar can increase the effectiveness of rhizobacterial nodulation with plants due to increased aeration by biochar so that bacteria can survive longer in biochar pores to further infect roots. The provision of biochar can improve the microbial mutualistic relationship to support the development of cocoa pods.

CONCLUSIONS

Interaction between biochar 5 tons ha⁻¹ and Rhizobacteria 100 mL L⁻¹ showed the best result for chlorophyll a, chlorophyll b, and total chlorophyll. The interaction between biochar 5 tons ha⁻¹ and Rhizobacteria 150 mL L⁻¹ showed the best results for the number

of harvested pods per tree, bean weight per tree, stomatal opening area. The interaction between biochar 7.5 tons ha⁻¹ and Rhizobacteria 150 mL L⁻¹ showed the best results on the weight of 100 dry beans at water content 8%.

REFERENCES

- Abel, S.; A. Peters; S. Trinks; H. Schonsy; M. Facklam & G. Wessolek (2013). Impact of biochar and hydrocar addition on water retention and water repellency of sandy soil. *Geoderma*, 202–203, 183–191.
- Antonius, S.; D.S. Rozy; N. Yulia & K.D. Tirta (2018). Manfaat pupuk organik hayati, kompos dan biochar pada pertumbuhan bawang merah dan pengaruhnya terhadap biokimia Tanah pada percobaan pot menggunakan tanah Ultisol. *Jurnal Biologi Indonesia*, 14(2), 243–250.
- Ardiansyah, M.; N. Budi & S. Khalimatus (2022). Estimasi kadar klorofil dan N daun jagung menggunakan *chlorophyll content index*. *Jurnal Ilmu Tanah dan Lingkungan*, 24(2), 53–61.
- BPS (2021). *Statistik Perkebunan Provinsi Sulawesi Selatan 2019–2021*. Badan Pusat Statistik, Sulawesi Selatan.
- Dharmadewi, A.A.I.M. (2020). Analisis kandungan klorofil pada beberapa jenis sayuran hijau sebagai alternatif bahan dasar food supplement. *Kemasains: Jurnal Edukasi Matematika dan Sains*, 9(2), 171–177.
- Ditjenbun (2022). *Statistik Perkebunan Unggulan Nasional 2020–2022*. Direktorat Jenderal Perkebunan Kementerian Pertanian Republik Indonesia, Jakarta.
- Felania, C. (2017). *Pengaruh Ketersediaan Air terhadap Pertumbuhan Kacang Hijau (Phonaceolus radiatus)*. Fakultas MIPA Universitas Negeri Yogyakarta, Yogyakarta.
- Gumelar, A.I. & K.T. Yunus (2021). Pengaruh frekuensi pemberian pupuk organik cair dan takaran *Biochar* terhadap pertumbuhan dan hasil sawi pakcoy (*Brassica rapa L.*). *Jurnal Pertanian Konservasi Lahan Kering*, 6(1), 4–7.
- Husen, E.; S. Rasti & D.H. Ratih (2006). *Rhizobakteri Pemacu Tumbuh Tanaman*. Balai Besar Penelitian dan Pengembangan Sumber Daya Lahan Pertanian, Jakarta.
- ICCO (2021). *ICCO Quarterly Bulletin of Cocoa Statistics*, 47(3) International Cocoa Organization.
- Irawan, T.B.; D.S. Liliek & N. Ann (2022). Respon pertumbuhan bibit kakao (*Theobroma cacao L.*) dengan pemberian berbagai konsentrasi PGPR (*Plant Growth Promoting Rhizobacteria*) akar kakao. *Ilmiah Hijau Cendekia*, 7(1), 7–18.
- Jabbarova, D.; S. Wirth; A. Kannepalli; A. Narimanov; S. Desouky & K. Davranov (2020). Co-inoculation of Rhizobacteria and biochar application improves growth and nutrients in soybean and enriches soil nutrients and enzymes. *Agronomy*, 10(8), 11–21.
- LKKT (2022). *Hasil Analisis Kandungan Hara Biochar*. Laboratorium Kimia dan Kesuburan Tanah, Departemen Ilmu Tanah, Fakultas Pertanian, Universitas Hasanuddin, Makassar.
- LKKT (2022.) *Hasil Analisis Sampel Tanah*. Laboratorium Kimia dan Kesuburan Tanah Departemen Ilmu Tanah, Fakultas Pertanian, Universitas Hasanuddin, Makassar.
- LKKT (2023). *Hasil Analisis Sampel Tanah*. Laboratorium Kimia dan Kesuburan Tanah, Departemen Ilmu Tanah, Fakultas Pertanian, Universitas Hasanuddin, Makassar, Indonesia.
- Nasaruddin; A. Ambo; P. Agil & N.J. Panga (2019). Response of cocoa leaves morpho-physiological characters to application of different microbes formulation. *IOP Conf. Series: Earth and Environmental Science*, 343, 1–7.

- Nurida, N.L.; A. Rachman & Sutono (2012). Potensi pembenah tanah *Biochar* alam pemulihan sifat tanah terdegradasi dan peningkatan hasil jagung pada typic kanhapluduts Lampung. *Jurnal Penelitian Ilmu-Ilmu Kelaman: Buana Sains*, 12(1), 67–94.
- Osorio, S.; R. Yong-Ling & R.F. Alisdair (2014). An update on source-sink carbon partitioning in tomato. *Review Article Frontiers in Plant Science*, 5, 1–11.
- Puslitkoka (2004). *Panduan Lengkap Budidaya Kakao*. Agromedia Pustaka, Jakarta.
- Ren, H.; B. Huang; V. Fernandez-Garcia; J. Miesel; L. Yan & C. Lv (2020). Biochar and Rhizobacteria amendments improve several soil properties and bacterial diversity. *Microorganisms*, 8(4), 1–17.
- Sari, H.P.; I. Mohamad; W. Libria & R. Tri (2021). Pengaruh lama penggenangan terhadap pertumbuhan beberapa varietas tanaman tomat (*Lycopersicon esculentum*). *Jurnal Agriekstensi*, 20(1), 16–20.
- Sobari, I.; M. Herman & Saefuddin (2014). Budidaya kakao berwawasan konservasi. *Inovasi Teknologi Bioindustri Kakao*, 57–68.
- Suharja & Sutarno (2009). Biomass, chlorophyll, and nitrogen content of leaves of two chilli pepper varieties (*Capsicum annum* L.) in different fertilization treatments. *Nusantara Biosciences*, 1, 9–16.
- Vanek, S.J.; J. Thies; W. Bing; H. Kelly & L. Johannes (2016) Pore-size and water activity effects on survival of *Rhizobium tropici* in biochar inoculant carriers. *Journal of Microbial Biochemistry Technology*, 8(4), 296–306.
- Wang, J.; N. Spurthi; K. Karen & M. Ra (2013). Carbon partitioning in sugarcane (*Saccharum* species). *Mini Review Article Frontiers in Plant Science*, 4, 1–6.
- Yu, G-H. & Y. Kuzyakov (2021). Fenton chemistry and reactive oxygen species in soil: Abiotic mechanisms of biotic processes, controls and consequences for carbon and nutrient cycling. *Earth-Science Reviews*, 214, 103–125.

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