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Growth improvement of immature quinine by applying coffee husk compost and biofertilizer

Abstract. The growth of immature *Cinchona ledgeriana* can be optimized by applying fertilizers to fill the nutritional needs of plants. Nutrients can be provided through the help of microorganisms derived from organic fertilizers and biofertilizers. This study aims to evaluate the effect of applying coffee husk compost and biofertilizer consortium on the growth of immature quinine plants. This research was conducted from November 2023 – January 2024 at Gambung Tea and Cinchona Research Center, Pasir Jambu District, Bandung Regency, West Java. The research used a randomized block design with four replications and six treatments, namely control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹); solid coffee husk compost 3 kg.plant⁻¹; liquid coffee husk compost 80 mL.L⁻¹; biofertilizer 10 mL.L⁻¹; solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹; and liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹. The results showed applying organic fertilizer from coffee husk waste and biofertilizer improved the growth of immature quinine plants. Combination of liquid organic fertilizer from coffee husk waste 80 mL.L⁻¹ water + biofertilizer 10 mL.L⁻¹ water produced a high increase in leaf area. Combination of solid organic fertilizer from coffee husk waste 3 kg. plants⁻¹ + biofertilizer 10 mL.L⁻¹ water showed the highest plant height increment. It implied the success of quinine improvement growth at the immature phase by applying coffee husk waste and biofertilizers.

Keywords: Compost · Immature plant · Microorganism · Nutrient

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Introduction

Cinchona sp. is one of the plantation plants that came from America. *Cinchona* alkaloids are composed of 4 main alkaloids: quinine, quinidine, cinchonine, and cinchonidine. *Cinchona* alkaloids have pharmacological activities such as antimalarial, anticancer, anti-diabetic, muscle anti-cramp, hair growth stimulant, anesthetic, and antipyretic (Hariyanti et al., 2023). The need for quina extraction from various industrial sectors causes the demand to increase every single year. This condition is inversely proportional to the condition of quinine plantations in Indonesia. Based on data from Badan Pusat Statistik (2019), over the past almost 25 years (1995-2018), the total production and area of domestic quinine plantations have continued to decline by 4.2% per year. Quinine plantations in Indonesia are threatened with extinction if this condition continues.

One of the efforts that can be made to prevent the extinction of quinine plantations in Indonesia is to carry out maximum maintenance through fertilization. The fertilizer needs of quinine plants vary, and the dose will continue to increase as the plant ages. Maxiselly et al. (2022) stated that immature quinine plants need rich nutrition to develop their vegetative growth. The purpose of fertilization is to add organic or inorganic nutrients to the soil so that the nutrients needed by plants can be fulfilled. Organic fertilizers can be used as an alternative to reduce chemical fertilizers. Agricultural waste from the plant cultivation process is a natural material that can be used as organic fertilizer, such as coffee husk waste.

Coffee husk waste is produced through dry processing and wet processing of coffee. According to Antunez, et al. (2024), the coffee processing process will produce coffee husk waste as much as 40-50% of the total processed coffee beans. Most coffee farmers in Indonesia do not process the waste; they just throw it away or directly burn the coffee husk waste. Coffee husk waste can be utilized as a source of organic matter and nutrients that can increase plant growth and improve soil structure. According to Novita, et al. (2018), coffee's outer husk contains nitrogen (N) 1.9%, phosphorus (P) 0.3%, and potassium (K) 3.6%. Nitrogen plays a role in increasing the vegetative growth of plants, phosphate plays a role in the enlargement and division of plant cell tissues, and potassium

plays a role in the process of transporting enzymes, minerals, and the continuity of photosynthesis (Thamrin et al., 2020).

The percentage of nutrients contained in organic fertilizers is low when compared to the nutrient content of inorganic fertilizers. The application can be combined with biofertilizers to maximize the function of organic fertilizers. Biofertilizers are fertilizers derived from living microorganisms that can produce nutrients and growth hormones derived from elements in the soil (Venkateshwarlu, 2008). According to Ahmed, et al. (2023), biofertilizers can partially replace the function of chemical fertilizers because biofertilizers can stimulate the growth of microorganisms that provide nutrients for plants. When organic fertilizers and biofertilizers are combined, they facilitate the availability of nutrients in the soil for plants. Therefore, this research aimed to evaluate the effect of applying coffee husk compost and biofertilizer, which contained nitrogen-fixing and phosphate-solubilizing bacteria on the growth of immature quinine plants.

Materials and Methods

This research was conducted from November 2023 – January 2024 at Gambung Tea and Kina Research Center, Pasir Jambu District, Bandung Regency, West Java, with an altitude of \pm 1300 masl for the morphological trait observation. Solid and liquid organic fertilizers are analyzed at the Soil Chemical and Plant Nutrition Laboratory, Agricultural Faculty, Universitas Padjadjaran.

The plant materials used are the 2-year-old immature quinine plant species *C. ledgeriana*. All of the quinine plants used as samples were of seed origin and had gone through the process of transplanting to the plantation. The soil layer at the research site is subsoil with ordo andisol. This study used 3 types of fertilizers, including inorganic fertilizers, biofertilizers, and organic fertilizers, with the essential ingredients of coffee husk waste. Two organic fertilizers are used: solid organic fertilizer and liquid organic fertilizer. The materials used included coffee husk waste, EM-4, brown sugar, water, Bion-UP consortium biofertilizer, and control fertilizers like urea, SP-36, and KCl. Tools needed to help the research process include meters, rulers, measuring cups, analytical scales, vectors, hand

counters, filters, chlorophyll meters, cutters, stationery, paddles, sprayers, plant labels, notebooks, clipboards, buckets, hoes, shovels, scissors, and documentation tools.

The research method used was a randomized block design with six treatment combinations. Each treatment was repeated four times, including A: control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹), B: solid coffee husk compost 3 kg.plant⁻¹, C: liquid coffee husk compost 80 mL.L⁻¹, D: biofertilizer 10 mL.L⁻¹, E: solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹, and F: liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹.

Measurements parameters: The parameters observed included an increase in plant height (cm) measured from the base of the stem to the growing point, stem diameter (mm) measured using a caliper, and the measurements start at the height of 3 cm above the ground, leaf total was counted manually using a hand counter and only a leaf that has fully opened that counted, leaf area (cm²) measurements were carried out on left that is fully open by taking photos of the leaves then the area is calculated using ImageJ, leaf chlorophyll index measured using a Digital Chlorophyll Meter which clamped on the leaves until the number appear on the monitor screen with units of CCI, last is stem bark thickness that measured using a caliper by scraping a little bark using a cutter. Observations for plant height, stem diameter, and leaf chlorophyll index were made every two weeks, the total leaves were measured every month, for leaf area and stem bark thickness were measured only at the beginning and end of the observation. Observations were made every two weeks from 2 to 12 weeks after treatment.

All data collected were subjected to variance analysis and continued with Duncan's multiple range test in SASM-Agri software to test the difference between treatments.

Results and Discussion

The results of this study indicate that the treatment of organic fertilizer applications from coffee husk compost and consortium biofertilizers significantly affect the increase in plant height and the average of leaf area in immature quinine plants. In stem diameter, number of leaves, leaf chlorophyll index, and stem bark thickness, the treatment of organic

fertilizers from coffee husk waste and biofertilizers had no significant effect.

Plant Height Increase (cm). The treatment of organic fertilizers and biofertilizers significantly affected the height increase of immature quinine plants at 12 weeks after treatment (WAT). Treatments C and E had a better effect on the height increase than the other treatments. Data on the measurement of growth parameters carried out at 2 WAT to 10 WAT showed that each treatment was not significantly different from the control (Table. 1). This means that organic fertilizers and biofertilizers can replace inorganic fertilizers because both can have similar effects. This is probably because liquid organic fertilizer (LOF), solid organic fertilizer (SOF), and biofertilizers are used to contain various nutrients, phytohormones, and PGPR needed by plants.

Table 1. The effect of coffee husk waste and biofertilizer application on the increase of plant height at 1, 2, and 3 MAT

Treatment	The Increase of Plant Height (cm)		
	1 MAT	2 MAT	3 MAT
A	2.42	3.38	17.33 c
B	2.48	3.50	19.18 bc
C	2.87	4.10	26.75 a
D	2.50	3.50	21.10 abc
E	2.85	4.18	26.58 a
F	2.67	3.87	25.50 ab

*Values followed by the same letter within each row are not different at the 0.05 level of significance according to the DMRT test

* MAT = Month After Treatment

*Treatment:

A : control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹)

B : solid coffee husk compost 3 kg.plant⁻¹

C : liquid coffee husk compost 80 mL.L⁻¹

D : biofertilizer 10 mL.L⁻¹

E : solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹

F : liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹

The organic content of SOF composting processes can also cause abundant plant nutrients. Coffee husk waste contained C-organic 37.2%, C/N 14.3, N 2.6%, and pH 5.5. This met the quality standards of Permentan No.261 of 2019: minimum C-Organic 15%, C/N 25, pH 4-9, and N 2% (Permentan, 2019).The liquid organic fertilizer comprises composting coffee husk waste containing nitrogen-fixing

bacteria and phosphate-solubilizing bacteria at 1.37×10^{11} CFU.mL⁻¹ and 1.87×10^{11} CFU.mL⁻¹ (Maxiselly et al., 2023). Nitrogen-fixing bacteria such as *Azospirillum* sp. and *Azotobacter* sp. and phosphate-solubilizing bacteria like *Pseudomonas* sp. and *Penicillium* sp. assist in the provision of fertilizer elements due to the N fixation of air and the dissolving of P from inorganic P (Kumar et al. 2021). Both bacteria also contain various phytohormones, including cytokinin, gibberellin, and Indole Acetic Acid (IAA) (Setiawati et al., 2023). Phytohormones can increase plant water and mineral uptake and stimulate plant growth and roots.

Stem Diameter Increase (cm). Based on the results of the F test at 95% trust level, applying biofertilizer and organic fertilizer of coffee husk waste on quinine plants showed results that were not significantly different from the control treatment (Table. 2). This shows biofertilizers and coffee husk waste can replace inorganic fertilizers. Biofertilizers and organic fertilizers contain various organic materials that can improve the physical, chemical, and biological characteristics of soil, such as increasing cation exchange capacity, water capacity, pH, and porosity and stimulating the growth of microorganisms on soil so that the absorption of nutrients by plants becomes more optimal (Santos et al., 2024).

Table 2. The effect of coffee husk waste and biofertilizer application on the increase of stem diameter at 1, 2, and 3 MAT

Treatment	The Increase of Stem Diameter (cm)		
	1 MAT	2 MAT	3 MAT
A	0.83	1.40	1.59
B	0.98	1.47	1.82
C	0.86	1.52	1.88
D	0.96	1.55	1.79
E	0.92	1.51	1.95
F	0.96	1.49	1.88

* MAT = Month After Treatment

*Treatment:

- A : control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹)
 B : solid coffee husk compost 3 kg.plant⁻¹
 C : liquid coffee husk compost 80 mL.L⁻¹
 D : biofertilizer 10 mL.L⁻¹
 E : solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹
 F : liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹

N-fixing and P-solubilizing bacteria in biofertilizers and organic fertilizers of coffee

husk waste can synthesize IAA, cytokinins, and gibberellins (Vikhe, 2014). IAA is a hormone that can stimulate plant growth and stem elongation and increase the elongation process and cell differentiation (Advinda et al., 2018). Gibberellin can stimulate the extension of stem internodes (Small et al., 2018). These hormones can spur plant growth and suppress phytopathogen (Reddy et al., 2014).

Number of Leaves. The analysis results with the F test at the 95% trust level showed that applying organic fertilizer and biofertilizer were not significantly different from the control treatment (Table. 3). This means biofertilizers or organic fertilizers can replace chemical fertilizers.

Table 3. The effect of coffee husk waste and biofertilizer application on the leaf total at 1, 2, and 3 MAT

Treatment	Leaf Total		
	1 MAT	2 MAT	3 MAT
A	2.69	1.25	1.42
B	2.53	1.19	1.45
C	2.96	1.28	1.44
D	2.33	1.15	1.38
E	2.65	1.09	1.35
F	2.53	1.24	1.42

* MAT = Month After Treatment

*Treatment:

- A : control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹)
 B : solid coffee husk compost 3 kg.plant⁻¹
 C : liquid coffee husk compost 80 mL.L⁻¹
 D : biofertilizer 10 mL.L⁻¹
 E : solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹
 F : liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹

Biofertilizers and organic fertilizers from coffee husk waste contain nitrogen-fixing and phosphate-solubilizing bacteria that can provide N and P elements through soil microbial activity. The liquid organic fertilizer used in this research contains 1.37×10^{11} CFU/ml N-fixing bacteria and 1.87×10^{11} CFU/ml P-solubilizing bacteria. Nitrogen increases the vegetative growth of plants, such as leaves and stems (Thamrin et al., 2020). According to Chaudhary et al. (2022), biofertilizers contain exopolysaccharides and phytohormones that can increase plant growth and help nutrient absorption. One of the hormones produced is gibberellin, which plays a role in cell division and enlargement (Gamalero et al., 2023). The research

results by Maria, et al. (2013) showed that gibberellin increases the growth of leaf number in plants through cell division at the tip of the crown and increases cell wall plasticity. The application of organic fertilizer and biofertilizer did not significantly affect the increase in the number of leaves of quinine plants, probably due to several factors, such as sample plants originating from seeds. According to Razaq, et al. (2015), generative propagation has high genetic variation with low productivity. The use of biofertilizers has a less significant impact on the increase in the number of leaves because genetics play a more substantial role in determining the number of leaves (Karamat et al., 2021).

Average Leaf Area. The DMRT results in (Table 4) showed that at 12 WAT, the SOF+BF and LOF+BF treatment significantly affect the increase of the average leaf area in immature quinine plants. Combining organic fertilizers with biofertilizers can increase fertilization efficiency in plants because the microorganisms in fertilizers have a role in plant growth, increasing plant yield and improving soil conditions. This is in line with the results of research by Maxiselly et al. (2023), namely, the composition of liquid and solid coffee husk combined with biofertilizers effectively increases the growth of immature Liberica coffee.

Table 4. The effect of coffee husk waste and biofertilizer application on the average leaf area at 3 MAT

Treatment	Average Leaf Area (cm ²)
	3 MAT
A	2.69 c
B	3.04 c
C	3.20 bc
D	3.72 ab
E	3.96 a
F	3.83 a

*Values followed by the same letter within each row are not different at the 0.05 significance level according to the DMRT test.

* MAT = Month After Treatment

*Treatment:

- A : control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹)
- B : solid coffee husk compost 3 kg.plant⁻¹
- C : liquid coffee husk compost 80 mL.L⁻¹
- D : biofertilizer 10 mL.L⁻¹
- E : solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹
- F : liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹

Coffee husk waste compost and biofertilizers used in treatments E and F are composting products that contain various macronutrients needed by plants, such as N, P, and K. Coffee husk waste contained C-organic 37.2%; C/N 14.3; N 2.6%; and pH 5.5. Nitrogen is required by plants because it functions as a constituent of proteins, amino acids, and chlorophyll pigment components that are important in the photosynthesis process (Fathi, 2022). This is in line with the results of research by Olowoboko et al. (2017); the nitrogen content in fertilizer can increase the leaf diameter more significantly.

Both biofertilizers and coffee husk organic fertilizers contain Nitrogen-fixing bacteria that play a major role in providing nitrogen elements. N-fixing bacteria such as *Azotobacter* sp. have a complete mechanism as a potential microbe that can stimulate plant growth by providing nitrogen, phytohormones, and antifungals (Hala et al., 2023). *Azotobacter* sp. has a major role in supplying N for plants in the form of ammonium (NH₄⁺) through the mechanism of nitrogen fixation (Aasfar, 2021). Another N-fixing bacterium, *Acinetobacter* sp., is also a PGPR that increases chlorophyll content in plants (Suzuki et al., 2014).

Leaf Chlorophyll index. Based on the results of the F test at the 95% trust level, the treatment of organic fertilizers and biofertilizers did not significantly affect the increase in leaf chlorophyll index of immature cinchona plants at 2 until 12 WAT (Table. 5). Among all treatments, SOL and biofertilizer treatments had the most positive effect. This is thought to be because both SOL and biofertilizers contain nitrogen-fixing bacteria that can synthesize nitrogen elements, so the N needs of plants can be fulfilled.

According to Sampson, et al. (2003), high or low chlorophyll content is directly proportional to the availability of nitrogen elements that play an important role in photosynthesis. The higher the availability of nitrogen for plants, the higher the chlorophyll content in the leaves so that the photosynthesis process takes place quickly. N-fixing bacteria in biofertilizers and organic fertilizers from coffee husk waste helps provide N for plants. In this study, the role of biofertilizers and organic fertilizers in increasing the leaf chlorophyll index has not had a significant effect. This is thought to be because organic fertilizers are slow to release or

decompose nutrients slower than chemical fertilizers (Shaji et al., 2021). The insignificant results are also thought to be due to the value of nutrients in LOF and biofertilizer, which are still relatively low, so the nutrients absorbed by plants are not maximized.

Table 5. The effect of coffee husk waste and biofertilizer application on the leaf chlorophyll index at 1, 2, and 3 MAT.

Treatment	Leaf Chlorophyll Index (CCI)		
	1 MAT	2 MAT	3 MAT
A	4.33	4.98	4.94
B	4.05	5.05	5.32
C	4.05	4.78	5.37
D	3.68	4.61	4.92
E	3.82	4.78	4.96
F	3.51	4.76	5.34

* MAT = Month After Treatment

*Treatment:

- A : control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹)
 B : solid coffee husk compost 3 kg.plant⁻¹
 C : liquid coffee husk compost 80 mL.L⁻¹
 D : biofertilizer 10 mL.L⁻¹
 E : solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹
 F : liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹

Stem Bark Thickness. The results of the F test, at 95% trust level, showed that the treatment of coffee husk waste organic fertilizer and biofertilizer did not significantly affect the increase in quinine bark thickness (Table. 6). SOF application combined with biofertilizers showed relatively higher growth in bark thickness compared to the control treatment. The positive increase is due to the role of nutrients and phytohormones produced by applying organic fertilizer and biofertilizer.

Nitrogen-fixing bacteria in biofertilizers and organic fertilizers from coffee husk waste function to fix N₂ and convert it into a form available for plants. The bacteria can produce growth substances such as IAA, auxin, and gibberellin (Setiawati et al., 2023). The stem bark's thickness is closely related to the plant stem's cambium content. According to Rüscher, et al. (2021), auxin can stimulate the development of vessel tissue and encourage cell division in the vessel cambium. The phosphate-solubilizing bacteria in biofertilizers and organic fertilizers function to release organic acids that can chelate Fe, Al, Mg, and Ca, so the bound

phosphorus is available for plants (Boraste et al., 2009).

Table 6. The effect of coffee husk waste and biofertilizer application on the stem bark thickness at 3 MAT.

Treatment	Stem Bark Thickness
	3 MAT
A	0.43
B	0.45
C	0.45
D	0.40
E	0.45
F	0.50

* MAT = Month After Treatment

*Treatment:

- A : control (Urea 17 g.plant⁻¹ + SP36 8 g.plant⁻¹ + KCl 4 g.plant⁻¹)
 B : solid coffee husk compost 3 kg.plant⁻¹
 C : liquid coffee husk compost 80 mL.L⁻¹
 D : biofertilizer 10 mL.L⁻¹
 E : solid coffee husk compost 3 kg.plants⁻¹ + biofertilizer 10 mL.L⁻¹
 F : liquid coffee husk compost 80 mL.L⁻¹ + biofertilizer 10 mL.L⁻¹

The amount of organic matter in organic fertilizers and biofertilizers is limited, and their slow-release characteristic causes the fulfillment of nutrients in plants to be less than optimal. SOF is less effective than LOF because fertilizers in the soil must be absorbed through roots, which takes longer than LOF applied directly to leaves. (Panjaitan, 2023). Applying coffee husk waste and biofertilizers potentially lowers the high need for inorganic fertilizers during the immature period of quinine

Conclusion

Applying coffee husk waste and biofertilizer significantly improved the growth of immature quinine as indicated by plant height and leaf area variables at 3 MAT. However, the application of organic fertilizer from coffee husk waste and biofertilizer did not significantly affect the increase in stem diameter, number of leaves, leaf chlorophyll index, and stem bark thickness of immature quinine plants. These results implied the potential recommendation to apply organic fertilizer from coffee husk waste and biofertilizer for compensating the high demand for inorganic fertilizers.

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