Bio-Priming of Seeds with Endophytic Bacterial Suspension and Compost Application for Enhancing Growth Rate of Liberica Coffee (*Coffea liberica* L.)

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Abstract
Liberica coffee (*Coffea liberica* L.) has high adaptation advantages to marginal land conditions and tropical climate but remains rarely cultivated commercially due to challenges in the initial nursery process which requires a relatively long duration. Research about bio-priming of seeds with endophytic bacterial suspension and compost application for enhancing growth rate of liberica coffee aimed to examine the effect of endophytic bacterial suspension immersion and compost application on growing medium on the growth rate of liberica coffee as an effort to develop environmentally friendly and sustainable nursery technology. This research employed a completely randomized design (CRD) with two factors: immersion duration (0, 6, 8, and 10 hours) and compost dose (0, 100, 150, and 200 grams), with 16 treatment combinations and five replications, followed by analysis of variance (ANOVA) and Duncan's test. Treatment A1 (6-hour immersion) produced the best plant height of 17.23 cm at 4 months after planting (MAP), representing a 36% increase compared to control. Treatment A3 (10-hour immersion) produced the highest number of leaves at 11.12 leaves at 4 MAP, showing a 12% increase compared to control. Treatment A3 also produced the highest leaf area of 27.67 cm² at 4 MAP, representing a 37% increase compared to control, while compost treatment M2 (150 g) produced optimal results with leaf area of 27.54 cm². The combination of endophytic bacterial suspension biopriming with compost application creates an optimal synergistic system for liberica coffee seedling growth, with 6 hours immersion duration being optimal for plant height while 10 hours immersion was optimal for leaf formation and expansion, and both parameters demonstrated the capacity of endophytic bacteria to stimulate vegetative growth through phytohormone production mechanisms.

Keywords: *Coffea Liberica*, *Seed Biopriming, Endophytic Bacteria*

Introduction

Coffee is a strategic agricultural commodity possessing high economic value and playing an important role in Indonesia's national economy. Indonesia is recorded as the fourth-largest coffee producer and exporter in the world after Brazil, Vietnam, and Colombia, with total production in 2024 reaching 807,580 tons, marking the highest achievement in the past decade (BPS., 2024). Among the various types of coffee cultivated, liberica coffee (*Coffea liberica*) is still rarely cultivated commercially and contributes less than 1.5% of global coffee production, despite having high adaptation advantages to marginal land conditions and tropical climate (Rahmawati *et al*., 2024).

The primary challenge in Liberica coffee development lies in the initial nursery process, which requires a relatively long duration. Liberica coffee seeds possess a hard seed coat and undergo dormancy with germination time lasting 8 until 12 weeks under optimal conditions, or even up to three months under certain circumstances. These conditions require special treatments to accelerate and improve germination quality as well as seedling growth. One promising approach is through the seed biopriming technique using beneficial bacterial suspension combined with growing medium optimization. The technique of seed biopriming with beneficial microorganisms has been proven capable of enhancing germination, seedling vigor, and tolerance to both biotic and abiotic stress in various agricultural crops (Tambunan 2023; Mahmood *et al*., 2016).

Endophytic bacteria are microorganisms that live and associate within plant tissue without causing disease symptoms to their host. Research demonstrates that endophytic bacteria from coffee plants have dual potential as biological control agents and plant growth promoters through mechanisms of phytohormone production such as Indole Acetic Acid (IAA), nitrogen fixation, and phosphate solubilization (Setiawan & Warsito 2025). Warsito *et al*. (2024) have isolated endophytic bacteria from stems of Arabica coffee plants and demonstrated their effectiveness in inhibiting pathogen growth and producing IAA at the highest concentration reaching 66.36 ppm, which plays a role in stimulating plant growth.

Beyond seed treatment, growing medium quality is also a crucial factor in supporting coffee seedling growth. The use of compost as an organic material in growing medium has been proven capable of improving the physical, chemical, and biological properties of soil, thereby enhancing coffee seedling growth (Kadha *et al.,* 2023). Research by Muliasari (2016) demonstrated that the application of 25% inorganic fertilizer dose combined with 75% organic compost fertilizer from cattle manure represents the best combination for Arabica coffee seedling growth with 75% shade intensity. Coffee hull compost is also reported to effectively enhance seedling growth due to its high potassium (K) content of 1.3%, which significantly influences seedling growth until reaching the distribution-ready standard height of 15 cm.

The state of the art of this research is an innovative combination between the technique of endophytic bacterial suspension immersion (seed biopriming) with compost application on growing medium, specifically applied to liberica coffee seeds in the initial nursery stage. This approach integrates current encapsulation technology and biofertilizer formulation capable of enhancing bacterial viability and effectiveness in promoting plant growth. Therefore, this research aims to examine the effect of endophytic bacterial suspension immersion and compost application on growing medium on the growth rate of liberica coffee as an effort to develop environmentally friendly and sustainable nursery technology.

Research Methodology

2.1 Seed Immersion with Endophytic Bacterial Suspension

Endophytic bacteria used from Warsito *et al*. (2024). An endophytic bacterial suspension solution was obtained by adding 10 mL of 0.9% physiological NaCl solution to one petri dish containing bacterial colonies, then homogenized using a triangular stirring rod. Coffee seeds were immersed in the suspension with immersion duration variations of 6 hours, 8 hours, and 10 hours. During the immersion process, the container was covered using aluminum foil to maintain sterile conditions.

2.2 Growing Medium Preparation

The growing medium consisted of topsoil, compost fertilizer, and rice husk charcoal, with each component equally balanced at 25%. All materials were placed in 60 polybags. Before use, the medium had to be sterilized by steaming in a drum or large pan at 150° C for 8 hours and repeated for two consecutive days to reduce harmful microorganisms.

2.3 Data Analysis

This research used a completely randomized design (CRD) with two factors, with 16 treatment combinations and five replications. The first factor was immersion duration of suspension (A0, A1: 6 hours, A2: 8 hours, A3: 10 hours), and the second factor was compost type (M0, M1: 100 g, M2: 150 g, M3: 200 g). The obtained data were analyzed using analysis of variance (ANOVA) followed by Duncan's test to determine significant differences among treatments. The observed parameters included plant height (cm); number of leaves (leaves); leaf area (cm^2).

Results

3.1 Plant Height Parameter

From observations conducted on plant height over 4 months after planting (MAP), the following observation data were obtained.

Table 1. Observations of Plant Height Parameter

Treatment	Heigh Average		
Immersion duration	**2 MAP**	**3 MAP**	**4 MAP**
A0	8.13a	9.89a	12.67a
A1	9.12a	11.88ab	17.23b
A2	8.82ab	10.54ab	15.14b
A3	8.67b	11.12b	15.35c
Growing Medium			
M0	8.56a	10.62a	12.33a
M1	8.23b	9.77a	11.25a
M2	7.41b	9.87ab	13.66ab
M3	8.23b	10.33ab	11.17b

Research results demonstrate that the seed immersion treatment of liberica coffee in endophytic bacterial suspension had a significant effect on the plant height parameter. The A1 treatment (6-hour immersion) produced the best plant height of 17.23 cm at 4 MAP, increasing by 36% compared to control (12.67 cm). The increase in plant height under this biopriming treatment indicates that the endophytic bacteria used were capable of stimulating vegetative

growth of liberica coffee seedlings through phytohormone production mechanisms, particularly Indole Acetic Acid (IAA).

These findings are consistent with research by Fiodor *et al.* (2023) reporting that seed biopriming with plant growth-promoting bacteria (Plant Growth Promoting Bacteria/PGPB) significantly increased seedling height and coleoptile vigor in various plants. The mechanism of plant height increase through biopriming occurs because endophytic bacteria produce IAA which plays a role in cell elongation and stem extension. According to Etesami and Glick (2024), bacterial IAA enhances plant growth by stimulating cell division and elongation, increasing protein synthesis, and activating gene expression related to growth. This is supported by research from de Souza et al. (2025) demonstrating that IAA-producing bacteria significantly increase the growth and nutrition of coffee seedlings.

Research by Warsito *et al.* (2024) on Arabica coffee plants showed that application of microgranules from IAA-producing endophytic bacteria at concentration of 66.36 ppm was capable of notably stimulating vegetative growth. The 6-hour immersion duration in treatment A1 is believed to be the optimal time for absorption and colonization of endophytic bacteria on liberica coffee seed coat without causing toxic effects or hormone excess. Research by Shaffique *et al.* (2023) explains that biopriming affects coleoptile length through the phenomenon of prime-omics, wherein bioprimed seeds possess greater root and shoot elongation as well as fresh and dry biomass compared to the control group.

Interestingly, longer immersion durations (A2: 8 hours and A3: 10 hours) showed lower plant height growth compared to A1, although still better than control. This phenomenon is likely caused by saturation or even inhibitory effects resulting from excessive IAA concentration. Sun *et al.* (2023) explains that IAA at low to moderate concentrations promotes cell elongation, but at high concentrations can inhibit growth through induction of ethylene synthesis.

Compost application on growing medium also contributed to the increase in plant height. Treatment M2 (compost) produced plant height of 13.66 cm at 4 MAP, higher than the control medium. Research by Kadha *et al.* (2023) on coffee seedlings (*Coffea* sp.) reported that growing medium containing compost was capable of significantly increasing seedling growth rate because it improved the physical, chemical, and biological properties of the medium. Compost increases the availability of macro and micronutrients, improves soil structure, increases cation exchange capacity (CEC), and enhances the activity of beneficial soil microorganisms.

3.2 Number of Leaves Parameter

Observations of number of leaves were conducted starting from 2 months after planting (MAP) until 4 months of age.

Table 2. Observations of Number of Leaves Parameter of Liberica Coffee

Treatment	Number of Leaves		
Immersion duration	**2 MAP**	**3 MAP**	**4 MAP**
A0	4.13a	6.13a	9.89a
A1	4.11a	6.12b	10.88ab
A2	4.65a	6.82b	10.54b
A3	4.24a	6.67c	11.12c

Growing Medium			
M0	4.56a	6.56a	10.62a
M1	4.71a	6.23b	10.77a
M2	4.34ab	6.41b	10.87ab
M3	4.11b	6.23bc	10.33ab

The number of leaves parameter showed different responses to immersion duration compared to plant height. Treatment A3 (10-hour immersion) produced the highest number of leaves at 11.12 leaves at 4 MAP, increasing by 12% compared to control (9.89 leaves). This difference in response indicates that the mechanism of leaf formation and stem elongation is influenced by different physiological pathways in responding to biopriming.

Leaf formation is a complex process involving the activity of shoot apical meristem and is regulated by the balance of several phytohormones, particularly auxin, cytokinin, and gibberellin. Research by Suriani *et al.* (2025) on Robusta coffee plants demonstrated that rhizobacterial consortium capable of producing IAA, nitrogen fixation, and phosphate solubilization significantly increased number of leaves and vegetative growth of coffee. Endophytic bacteria not only produce IAA but also can produce cytokinin which plays an important role in cell division and formation of lateral shoots and new leaves (Phuong *et al.,* 2025).

Longer immersion duration (10 hours) in treatment A3 likely allowed more maximal colonization of endophytic bacteria on seed tissue, thus enabling more intensive production of endogenous phytohormones during the seedling growth phase. Research by Purwanto et al. (2022) demonstrated that biopriming with rhizobacteria increased seed vigor index by up to 19.6% and promoted early plant vegetative growth. Nasution *et al.* (2025) also reported that endophytic bacteria from Arabica coffee roots producing IAA up to 291.64 ppm at pH 7.5 were capable of significantly increasing plant growth and number of leaves.

Treatment M0 (control) produced the highest number of leaves (10.62 leaves) compared to other media, although the difference was not substantial compared to compost media. This indicates that leaf formation is more influenced by biopriming factors compared to growing medium composition. However, research by Yunita *et al*. (2025) demonstrated that compost can increase total soil nitrogen which correlates positively with number of leaves ($r = 0.48$) and chlorophyll content in coffee plants. Nitrogen is an essential component in chlorophyll and protein synthesis required for formation of new leaves.

The interaction between biopriming and nutrient availability from compost creates optimal conditions for vegetative growth. Research by Takala *et al.* (2025) demonstrated that application of coffee hull compost at 7.5 t ha^{-1} combined with 50% inorganic fertilizer was capable of increasing plant height and number of primary branches in coffee plants. This combination not only increases nutrient availability but also improves soil structure and biological activity that supports plant growth.

3.3 Leaf Area Parameter

Leaf area observations were conducted only once when plants were 4 months old. Leaf area was calculated by measuring leaf length and width multiplied by the leaf area constant for coffee (0.747).

Table 3. Observations of Leaf Area Parameter of Liberica Coffee

Treatment	Leaf Area (cm^2)
Immersion duration	**4 MAP**
A0	20.13a
A1	25.11a
A2	25.53a
A3	27.67a
Media Tanam	
M0	26.21a
M1	26.23a
M2	27.54b
M3	26.34b

Leaf area is an important parameter that directly correlates with the photosynthetic capacity of plants. Research results demonstrate that treatment A3 (10-hour immersion) produced the highest leaf area of 27.67 cm^2 at 4 MAP, increasing by 37% compared to control (20.13 cm^2), although statistically not yet showing significant differences. This increase in leaf area is very important because it directly affects the plant's ability to capture light for photosynthesis process and biomass accumulation.

The mechanism of leaf area increase through biopriming with endophytic bacteria involves several physiological pathways. First, IAA production by endophytic bacteria stimulates cell expansion through cell wall loosening mediated by expansin proteins. Recent research by Iraji *et al.* (2025) demonstrates that IAA application increased plant length by 30.26%, fresh weight by 36.37%, and dry weight by 15.78% through increased cell elongation. Second, endophytic bacteria can also enhance nutrient uptake, particularly nitrogen and phosphorus, which are essential for chlorophyll synthesis and leaf tissue formation. Research by Pratiwi *et al.* (2020) on coffee endophytic bacteria showed that isolates capable of dissolving phosphate and fixing nitrogen produced better leaf growth.

Compost treatment M2 also provided the best results for leaf area with a value of 27.54 cm^2. Compost plays a role in increasing nutrient availability, particularly potassium which plays an important role in regulating stomata, cell turgor pressure, and leaf expansion. Research on Robusta coffee seedlings by Kadha and Neonbeni (2025) demonstrated that growing medium with composition soil:charcoal husk:compost (1:2:3) produced the largest plant height (13.65 cm) and stem diameter (5.57 mm) because compost improved aeration, water retention, and availability of macronutrients such as nitrogen, phosphorus, and potassium.

The combination between biopriming and compost application creates optimal synergy for leaf area development. Research by Ho *et al*. (2022) explains that compost increases soil pH, porosity, moisture, aggregate stability, organic content, and P and K content, while reducing bulk density. These favorable physicochemical conditions of the medium facilitate better root growth, thereby increasing water and nutrient uptake, which ultimately supports leaf area expansion. Miháliková *et al*. (2025) also reported that surface compost application increased soil organic matter content by 27.8 - 58.1%, saturated water content by 5.3 - 11.0%, and soil pH, all contributing to better plant growth.

The larger leaf area in seedlings undergoing biopriming is also related to increased chlorophyll content. Research by Gabuya *et al*. (2024) on Robusta coffee seedlings showed that optimal growth conditions increase chlorophyll content and photosynthesis efficiency. Yunita et al. (2025) reported a positive correlation between total soil nitrogen and chlorophyll content ($r = 0.71$) in coffee plants receiving compost. Higher chlorophyll content enables leaves to

conduct photosynthesis more efficiently, producing more photosynthates that can be used for further plant growth and development.

3.4 Synergistic Mechanism of Biopriming and Compost Application

The interaction between seed biopriming with endophytic bacteria and compost application on growing medium creates a synergistic growth system. Endophytic bacteria colonized on seeds through the biopriming process will remain active during the seedling growth phase and interact with soil microbiota enriched by compost. Compost not only functions as a nutrient source but also as a substrate supporting soil microbiological activity. Research by Wibowo *et al.* (2025) explains that application of compost and biochar can increase carbon sequestration in soil, provide water and nutrients, and improve plant growth. Growing medium rich in organic material from compost provides a conducive habitat for endophytic bacteria to develop and express plant growth-promoting characteristics such as IAA production, phosphate solubilization, and nitrogen fixation.

The optimal immersion duration (6 hours for plant height and 10 hours for number of leaves and leaf area) indicates that different growth parameters require different levels of bacterial colonization. Research by Kong *et al.* (2024) explains that seed priming enhances vigor by accelerating Î±-amylase enzyme activity and increasing total soluble sugar content that supports seed germination and early coleoptile growth. In the context of biopriming, optimal bacterial colonization ensures balanced phytohormone production to support various aspects of vegetative growth.

Recent research by Maxiselly *et al.* (2025) on liberica coffee showed that application of synthetic cytokinin can enhance coffee plant growth in the immature plant phase. This reinforces the concept that proper hormonal balance, both from exogenous sources (biopriming) and endogenous sources (plant production), is very important for growth optimization. The combination of biopriming with bacteria producing various phytohormones and compost application improving growing medium conditions creates ideal conditions for vigorous and high-quality liberica coffee seedling growth.

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