

Non-Toxic Effects of Forest Soil-Derived Microbial Inoculants on the Growth of Kepok Tanjung Banana Seedlings during the Ex Vitro Phase

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Abstract

Kepok tanjung banana (*Musa paradisiaca* var. *balbisiana*) is a priority commodities that has challenges in conventional seedling development because it is susceptible to disease. This study aims to evaluate the effect of non-toxic microbial inoculants isolated from tropical rainforest soil on the growth of Kepok tanjung banana seedlings in tissue culture seedlings. The method used was an experiment with a completely randomized design (CRD) involving a control and various microbial inoculation treatments (bacteria, fungi, and microbial consortium). Results showed that treatments SP8 and SP3 (bacteria) gave the highest stem height, 88.5 cm and 87.0 cm, respectively, and the largest stem diameter (SP3: 27.2 mm). In addition, the highest leaf length was found in SP8 (49.0 cm) with greener leaf color (score 5). All microbial treatments showed non-toxic results, with media pH remaining stable (6.3-6.6). The bacterial and fungal consortium (KJB) treatment, showed better results than single inoculants in improving nutrient availability and pathogen control. This research supports sustainable agriculture and contributes to the SDGs, particularly SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production) with an environmentally friendly solution for the efficient and sustainable propagation of banana seedlings.

Keywords: Microbial Inoculant, Kepok Tanjung Banana, Bacterial and Fungal Consortium, Tissue Culture, Sustainable Agriculture

1. Introduction

Kepok Tanjung banana (*Musa paradisiaca* var. *balbisiana*) is one of the important local banana cultivars in Indonesia due to its high economic value and wide utilization for food and agro-industry. The increasing demand for high-quality planting material has encouraged the use of tissue culture techniques as an efficient method for mass propagation. However, conventional seedling production of Kepok Tanjung banana is often constrained by low uniformity, poor seedling quality, and vulnerability to pests and diseases (Febmita & Putri, 2023). Tissue culture provides advantages in producing uniform and disease-free banana seedlings in a relatively short time. Nevertheless, one of the major bottlenecks in banana tissue culture lies in the post-culture phase, particularly during acclimatization and nursery ex vitro stages. At this stage, plantlets frequently experience high mortality rates due to sudden environmental changes, weak root systems, and limited nutrient absorption capacity. Therefore, improving the quality of the growing medium during acclimatization is crucial to enhance seedling survival and vegetative growth.

In recent years, the use of microbial inoculants as decomposing agents in growing media has received increasing attention, particularly during the acclimatization and ex vitro nursery stages of tissue-cultured banana seedlings. Soil microorganisms, including bacteria and fungi, play crucial roles in improving the physical structure of growing media, accelerating organic matter decomposition, and enhancing nutrient availability during plantlet transition from in vitro conditions to the external environment (Putri et al., 2024). The presence of beneficial microbial consortia further supports seedling establishment by enhancing tolerance to environmental stress and suppressing soil-borne pathogens, thereby improving acclimatization success and early vegetative growth under nursery conditions.

Microbial consortia composed of genera such as *Bacillus*, *Pseudomonas*, and *Trichoderma* have been widely reported to promote plant growth through synergistic interactions, including organic matter mineralization, nutrient mobilization, and biological disease control (Xiang et al., 2023). These functions are particularly relevant for tissue-cultured banana seedlings, which require a stable and biologically active growing medium during the ex vitro nursery phase. In addition, microbial inoculation has been shown to enhance plant resilience to unfavorable environmental conditions and improve resistance to diseases, contributing to more sustainable crop management practices (Kumar et al., 2024).

Despite the growing recognition of microbial inoculants, research on banana cultivation remains largely limited to general plant growth responses or preliminary assessments of microbial toxicity. Studies specifically addressing non-toxic microbial consortia compatible with tissue-cultured banana seedlings during

acclimatization and nursery ex vitro stages are still scarce (Chen et al., 2022). Moreover, the potential of microbial inoculants derived from forest soil ecosystems known for their high microbial diversity and functional stability has received little attention for this commodity. The limited availability of studies exploring forest soil-based microbial consortia highlights a significant research gap, particularly regarding their role as organic matter decomposers and soil conditioners in banana seedling production systems. Addressing this gap is essential for developing environmentally friendly and sustainable alternatives to synthetic inputs in banana nursery management. Therefore, This study aimed to evaluate the effects of non-toxic microbial inoculants derived from forest soil on the growth performance of tissue-cultured kepok tanjung banana seedlings during the ex vitro nursery stage. The study assessed the influence of microbial inoculation on seedling vegetative growth and growing medium characteristics, as well as its potential role in supporting sustainable and environmentally friendly banana seedling production.

2. Materials and Methods

This study used a Completely Randomized Design (CRD) experimental design with 9 treatments and 3 replicates for each treatment. The treatments applied included control and various microbial inoculants isolated from the soil of the Biology Education and Research Forest of Andalas University, with treatment details as follows: Control, SP1, SP2, SP3, SP6, SP8, KB (Bacterial Consortium), KJ (Fungal Consortium), and KJB (Fungal Bacterial Consortium).

Tissue cultured banana seedlings were obtained from the BRMP Tropical Fruit Research Center, Solok Regency, West Sumatra. The growing medium consisted of a mixture of peat soil and manure at a ratio of 2:1 (w/w) and was incubated for two weeks prior to use. Microbial inoculants were isolated from forest soil and comprised three bacterial isolates (BSP1, BSP2, BSP3) and two fungal isolates (JSP6, JSP8) with cellulolytic and lignocellulolytic potential. Bacterial isolates were identified based on morphological, biochemical, and 16S rRNA gene analyses, while fungal isolates were identified using macroscopic and microscopic colony characteristics. The inoculants were prepared at densities of approximately 10^8 CFU mL⁻¹ for bacteria and 10^6 spores mL⁻¹ for fungi. The equipment used included an incubator, pH meter, digital caliper, analytical balance, soil moisture meter, Leaf Color Chart, and a digital camera.

The procedure for conducting research, first is the preparation of microbial inoculants that have been isolated from forest soil. Furthermore, the preparation of planting media with a mixture of soil and manure in a ratio of 2:1. Second, seedlings obtained from tissue culture were transferred into polybags containing planting media, then microbial inoculants were added at a dose of 10 ml of each inoculant with the Soil-Borne Pathogen method gradually every one week for 4 weeks

after planting (mst). Observations were made every one week to assess the growth parameters of banana seedlings including: stem height (cm), stem diameter (mm): number of leaves (leaflets), leaf length (cm), leaf color: visually assessed on a scale of 1-5 with the Leaf Color Chart. In addition, measurements were made of the humidity of the growing media (%) and the pH of the growing media due to the application of microbial inoculants. Data were analyzed using Statistical Tool for Agricultural Research (STAR) to conduct Analysis of Variance (ANOVA). If the ANOVA results showed significant differences between treatments, the analysis was continued with Tukey's Honest Significant Difference (HSD) test to determine which treatment produced significant differences in banana seedling growth parameters.

3. Results and Discussion

Statistical analysis using ANOVA followed by Tukey's HSD test ($p < 0.05$) showed significant differences in several parameters, particularly stem height, stem diameter, leaf length, and leaf color. In contrast, the number of leaves, humidity, and the pH of

the growing media did not differ significantly among treatments. Observation data on the effect of inoculants on the growth of Kepok banana seedlings four weeks after planting are presented in Table 1. Statistical analysis using ANOVA and Tukey HSD test ($p > 0.05$) showed significant differences in several parameters, especially stem height, stem diameter, leaf length, and leaf color, while the number of leaves, humidity, and pH of the growing media were relatively not significantly different between treatments. Observation data on the effect of inoculants on the growth of Kepok tanjung banana seedlings after 4 weeks after planting can be seen in Table 1.

By evaluating the effect of microbial inoculants on the growth of Kepok tanjung banana seedlings including bacteria, fungi, and bacterial-fungal consortium applied to tissue culture-derived Kepok tanjung banana seedlings, the data in Table 1 shows that microbial inoculation has a different impact on several growth parameters, such as stem height, stem diameter, number of leaves, leaf length, leaf color, soil moisture, and soil pH. This finding is in line with the basic principle that microbial inoculation can increase the efficiency of plant growth, as well as have a non-toxic effect on plants at the same dosage level.

Table 1. Effect of Forest Soil Non-Toxic Microbial Inoculants and Soil Moisture and pH on the Growth of Kepok Tanjung Banana Seedlings: Comparison of Bacteria, Fungi, and Microbial Consortia

Treatment	Inoculant Type	Average Stem Height (cm)	Average Stem Diameter (mm)	Average Number of Leaves (strands)	Average Leaf Length (cm)	Leaf Color	Average Humidity (%)	Average pH
Control	-	85.0±4.25 ^{ab}	24.9 ± 1.24 ^{ab}	4 ± 0.20	40.0 ± 2.00	4.5±0.23 ^{ab}	82.7 ± 4.14	6.5 ± 0.33
SP1	Bacteria	79.0±3.95 ^{bc}	26.2 ± 1.31 ^{ab}	5 ± 0.25	41.0 ± 2.05	4 ± 0.20 ^b	75.0 ± 3.75	6.3 ± 0.32
SP2	Bacteria	76.5±3.83 ^c	26 ± 1.30 ^{ab}	5 ± 0.25	41.5 ± 2.08	4.5±0.23 ^{ab}	82.7 ± 3.14	6.4 ± 0.32
SP3	Bacteria	87.0±4.35 ^a	27.2 ± 1.36 ^a	5 ± 0.25	44.0 ± 2.20	5 ± 0.25 ^a	70.7 ± 3.53	6.3 ± 0.32
SP6	Bacteria	85.0±4.25 ^{ab}	26.9 ± 1.35 ^a	5 ± 0.25	47.5 ± 2.38	4.5±0.23 ^{ab}	77.7 ± 3.89	6.5 ± 0.33
SP8	Bacteria	88.5±4.42 ^a	23.9 ± 1.19 ^b	4 ± 0.20	49.0 ± 2.45	5 ± 0.25 ^a	77.7 ± 3.89	6.6 ± 0.33
KB	Bacteria Consortium	79.5±3.98 ^{bc}	24 ± 1.20 ^{ab}	5 ± 0.25	45.0 ± 2.25	4 ± 0.20 ^b	60.0 ± 3.00	6.6 ± 0.33
KJ	Fungi Consortium	79.5±3.98 ^{bc}	23.5 ± 1.18 ^b	5 ± 0.25	42.5 ± 2.12	4 ± 0.20 ^b	75.0 ± 3.75	6.5 ± 0.33
KJB	of Bacteria and Fungi	85.5±4.28 ^{ab}	23.2 ± 1.16 ^b	5 ± 0.25	44.5 ± 2.23	5 ± 0.25 ^a	58.3 ± 2.91	6.6 ± 0.33

Notes: Values are presented as mean ± standard deviation (SD) based on three replications ($n = 3$). Means followed by different superscript letters within the same column indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

In the stem height parameter, SP3 and SP8 treatments containing bacteria gave significant results, with an average stem height of 87.0 cm and 88.5 cm, respectively. This treatment shows that microbes can stimulate the vegetative growth of banana seedlings. This increase in stem height is most likely due to the

ability of microbes to increase the availability of nutrients needed by plants, as described by Hajji-Hedfi et al., (2025) which confirms that bacteria are known to increase the solubility of soil nutrients, which supports the absorption of nutrients by plant roots and can stimulate higher stem growth.

Furthermore, observations of stem diameter showed that the SP3 and SP6 treatments resulted in larger stem diameters, reaching 27.2 mm and 26.9 mm, respectively, compared to other treatments. This result suggests that microbial inoculation, particularly involving bacterial and fungal isolates, may contribute to improved stem development in tissue-cultured banana seedlings. Beneficial microorganisms are known to enhance nutrient availability, stimulate root development, and promote the production of growth-regulating substances, which indirectly support vascular tissue development and stem thickening. Similar findings were reported by Hasan et al. (2024), who demonstrated that bacterial inoculation enhanced stem diameter through improved nutrient uptake and structural tissue development. Previous studies have also shown that plant growth-promoting microorganisms can influence stem growth by facilitating nitrogen solubilization, phosphorus availability, and hormonal balance, thereby supporting more robust plant architecture. Hasan et al., (2024), who demonstrated that bacterial inoculation enhanced stem diameter through improved nutrient uptake and structural tissue development. Previous studies have also shown that plant growth-promoting microorganisms can influence stem growth by facilitating nitrogen solubilization, phosphorus availability, and hormonal balance, thereby supporting more robust plant architecture (Compant et al., 2019).

Analysis of the leaf number parameter showed no significant differences among treatments, with most treatments producing a similar number of leaves, ranging from four to five leaves. Although SP3 and SP8 treatments exhibited a slightly higher leaf number (five leaves), these differences were not statistically significant and therefore indicate a relatively uniform vegetative response among treatments. Similarly, observations on leaf length did not show a statistically significant effect of microbial inoculation. The SP8 treatment produced the longest leaves (49.0 cm); however, this result represents a trend rather than a significant response. Previous studies have suggested that microbial inoculation may influence leaf development through improved nutrient availability and chlorophyll formation Singh & Khan,(2024), Jiyang et al., (2022) but such effects were not pronounced under the conditions of this study.

Visual observations of leaf color using the Leaf Color Chart indicated that SP3 and SP8 treatments tended to produce slightly greener leaves (scale 5), suggesting better leaf vigor. Nevertheless, as this parameter was assessed qualitatively, the results should be interpreted as supportive indicators rather than definitive evidence of enhanced photosynthetic capacity. Weekly measurements of growing medium moisture showed variation among treatments, with SP2, SP6, and KJB exhibiting relatively lower moisture levels. This variation may be related to microbial activity influencing organic matter decomposition and water dynamics in the growing medium. However, since moisture differences were not the main focus of this study, these results are presented as supplementary observations.

Weekly observations of growing-medium moisture throughout the experimental period revealed significant differences among treatments. Treatments SP2, SP6, and KJB exhibited lower moisture levels compared with the other treatments, with average values ranging from 58.3% to 75%. This reduction in moisture was not detrimental, as the values remained within the optimal range for Kepok banana seedling growth and did not induce symptoms of drought stress. These conditions indicate increased water utilization within the growing medium, which is likely associated with microbial activity in decomposing organic matter and enhancing water uptake efficiency by the plant root system. This interpretation is supported by Singh et al., (2023) who reported that microbial inoculation can improve plant tolerance to drought conditions through enhanced root structure and increased water use efficiency, thereby enabling plants to maintain growth and survival under relatively lower soil moisture levels.

The improvement in soil pH quality observed in some treatments, such as SP3 and SP8, indicates that microbes can modulate soil pH to support plant growth. In general, soil pH was within the range of 6.3 to 6.6, which is an ideal pH range for banana plant growth. Research by Naz et al., (2022) revealed that a stable soil pH is essential in supporting microbial activity, which in turn increases the availability of nutrients required by plants.

The use of a microbial consortium consisting of a combination of bacteria and fungi, as applied in the KJB treatment, showed quite good results on the parameters of leaf length and stem diameter. This treatment shows that the microbial consortium can work more effectively in supporting plant growth. This is in line with findings from Timofeeva et al., (2023) , which showed that microbial consortia have greater potential to increase plant resistance to pathogens and improve nutrient absorption compared to single microbial inoculations.

Although single bacterial inoculation gave positive results, the use of microbial consortia combining bacteria and fungi showed more comprehensive results in supporting various aspects of plant growth. Research by Díaz-Rodríguez et al., (2025) also shows that different microbial combinations can complement each other in supporting plant growth, both in terms of increasing resistance to pathogens, nutrient utilization, and improving soil quality.

Microbial consortium treatments that combine bacteria and fungi show better results than single inoculants. This is due to a more complex symbiotic interaction between bacteria and fungi, which complement each other in supporting plant growth. Microbial consortia can increase nutrient availability through various mechanisms, such as bacteria that help dissolve phosphorus and fungi that play a role in pathogen control. Research by Díaz-Rodríguez et al., (2025) also shows that the use of microbial consortia is more effective in increasing plant resistance to pathogens and improving nutrient absorption compared to the use of single microbial inoculants. The

results of this study reinforce the understanding that the use of microbial consortia from forest soil, as reflected in the title "Non-Toxic Effects of Forest Soil-Derived Microbial Inoculants on Kepok tanjung banana Plant Growth," provides a more comprehensive and

environmentally friendly approach in improving the growth of Kepok tanjung banana seedlings. Visually, the difference between seedlings before inoculant application in Figure 1 and after inoculant application can be seen in Figure 2.



Figure 1. General appearance of tissue-cultured Kepok Tanjung banana seedlings before microbial inoculant application (0 weeks after planting) under ex vitro nursery conditions. Seedlings showed relatively uniform morphological characteristics prior to treatment application.



Figure 2. General appearance of tissue-cultured Kepok Tanjung banana seedlings four weeks after microbial inoculant application during the ex vitro nursery stage. The figure illustrates the overall growth condition of seedlings after treatment, while quantitative differences among treatments are presented and discussed based on statistical analysis (Tables 1).

4. Conclusion

This study demonstrates that non-toxic microbial inoculants isolated from forest soil can positively influence specific growth parameters of tissue-cultured Kepok tanjung banana seedlings. Treatment SP8 resulted in the highest stem height (88.5 cm) and leaf length (49.0 cm); however, its stem diameter was lower than that observed in treatments SP3 and SP6, indicating that different growth parameters responded differently to microbial inoculation. All treatments exhibited non-toxic effects, as evidenced by the relatively stable growing-medium pH values (6.3–6.6). These results suggest that the effectiveness of microbial inoculants is parameter-dependent rather than uniformly superior across all growth traits. Additionally, microbial consortia tended to enhance nutrient availability and potential pathogen suppression

compared to single inoculants, although their effects varied among growth variables. Overall, the findings highlight the potential of forest-derived microbial inoculants as environmentally friendly inputs for sustainable banana seedling production, supporting the objectives of SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production).

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