



Synergistic Effects of Photosynthetic Bacteria and Endophytes: A Novel Approach to Enhance Cayenne Pepper Productivity

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ABSTRACT

Cayenne peppers (*Capsicum frutescens*) are a valuable commodity contributing significantly to the economy of Sleman Regency. Despite their substantial economic potential, cayenne pepper farming practices in Sleman often lack integration of sustainable and eco-friendly agricultural principles. This research aims to investigate the potential of utilizing photosynthetic bacteria and endophytic bacteria to enhance the growth and yield of cayenne peppers in Sleman Regency. The study was conducted in Gondang Lutung Hamlet, Donoharjo Village, Ngaglik District, Sleman. A randomized complete block design (RCBD) was employed with a single factor: beneficial bacteria type, including P1 (PSB + Endophyte), P2 (PSB), P3 (Endophyte), and P4 (Control). The findings revealed that the combination of photosynthetic bacteria and endophytic bacteria had varying effects on cayenne pepper growth. The combination treatment significantly enhanced plant height, chlorophyll content, flower number, fruit number per plant, fresh fruit weight, production, productivity, and resistance to *C. capsici*, *C. gloeosporoides*, and *R. solanacearum*. Single PSB application improved plant growth attributes, while Endophyte application demonstrated potential for enhancing cayenne pepper resistance to pathogens. The study demonstrates the potential of utilizing beneficial bacteria, particularly the combination of PSB and Endophyte, to enhance cayenne pepper growth, yield, and disease resistance. This research provides valuable insights for promoting sustainable and eco-friendly cayenne pepper farming practices.

Keywords: ALA, Biostimulant, Hypersensitive Response, Plant-Microbe Interaction, Systemic Resistance

1. INTRODUCTION

Agriculture is not only a vital economic sector in Indonesia but also encompasses social, cultural, and environmental aspects. Thanks to its fertile natural conditions and the strong support of farming communities, agriculture has become the backbone in supporting numerous local families and communities. Cayenne pepper (*Capsicum frutescens*) has emerged as one of the leading commodities, making significant contributions to the national economy (Sodik & Winarti, 2023). The harvested area and production of cayenne peppers in Sleman Regency demonstrated a positive trend from 2020 to 2023, reaching 100,671 hectares and 1,506,762 tons, respectively. Compared to 2022, the cultivated area increased by 5.34%, while production showed decrease of 2.44% (Badan Pusat Statistik, 2024).

Despite its significant economic potential, cayenne pepper cultivation in Indonesia remains largely reliant on harmful practices. The most significant challenge faced in cayenne pepper cultivation is unstable production and excessive use of pesticides and chemical fertilizers according to (Latifah et al., 2019). This issue aligns with broader national concerns regarding agricultural sustainability and food safety. The indiscriminate application of agrochemicals contributes to soil degradation, water pollution, and residue contamination in food products, jeopardizing long-term agricultural productivity and public well-being (Sharma & Singhvi, 2017). To address these challenges, a paradigm shift towards sustainable agriculture is imperative. Incorporating eco-friendly strategies, such as the utilization of photosynthetic bacteria and endophytes, offers promising avenues for enhancing cayenne pepper production while mitigating environmental risks. These microbial agents have demonstrated the potential to improve plant growth, yield,

and disease resistance, thereby reducing the reliance on synthetic inputs. By adopting this innovative approaches, Indonesia can foster a more resilient and sustainable cayenne pepper industry, safeguarding the environment and ensuring the production of safe and high-quality crops for domestic consumption and export.

According to Du et al. (2022), photosynthetic bacteria possess a unique ability to collaborate with plants in enhancing photosynthesis, nutrient uptake, resistance to environmental stress, and soil quality improvement. Previous studies have demonstrated the significant potential of these bacteria in boosting plant growth and yield, including in food and horticultural crops. Avianto (2023) reported that foliar application of photosynthetic bacteria can enhance growth, chlorophyll content, and consumption index in green mustard plants. PSB application, particularly through pouring into growing media, significantly enhanced multiple growth parameters and yield in shallots (Avianto & Susila, 2024). In the study conducted by Hsu et al. (2021), an experiment was carried out involving the addition of photosynthetic bacteria to hydroponic nutrient solutions for the cultivation of green mustard, white mustard, and lettuce. The results demonstrated a significant increase in fresh plant weight and a decrease in nitrate content within plant tissues following the application of these bacteria. Furthermore, the number and fresh weight of tomato fruits were reported to increase after the application of photosynthetic bacteria (Du et al., 2022). In chili plants, photosynthetic bacteria can increase fruit production per plant and suppress the potential for *Phytophthora* blight (Luo et al., 2014).

On the other hand, endophytic bacteria have the ability to combat plant pathogens that cause diseases in chili plants, such as anthracnose, *Fusarium* wilt, and bacterial wilt (Kim et al., 2016; Muhammad et al., 2023; Yanti et al.,

2018, 2019). Endophytic bacteria are bacteria isolated from plant organs such as leaves, stems, roots, and even seeds. These bacteria live symbiotically within plant tissues without causing any disease to the plant (Jin et al., 2014). The combination of these two types of bacteria has the potential to enhance the growth, yield, and disease resistance of cayenne pepper. A novel aspect of this research is the concurrent utilization of photosynthetic bacteria and endophytes to enhance cayenne pepper cultivation. Previous research has primarily focused on the individual effects of these microbial groups. In contrast, this study aims to harness the potential synergistic benefits derived from their combined application to optimize crop performance. Therefore, this research aims to investigate in more detail the potential of utilizing photosynthetic bacteria to improve the growth and yield of cayenne pepper.

2. MATERIAL AND METHODS

2.1 Research Location and Preparation

A field experiment was conducted from August 2022 to May 2023 in Gondang Lutung Hamlet, Donoharjo Village, Ngaglik District, Sleman (7°41'55.1"S 110°23'08.5"E). The land utilized is classified as Entisol, characterized by poorly developed horizon layers and a predominance of sandy fractions. The land exhibits a neutral pH ranging from 6.74 to 6.89, with low total nitrogen content. Available phosphorus and potassium levels fall within the moderate category. The cayenne pepper variety ORI 212 was employed, along with photosynthetic bacteria and endophytic bacterial isolates BCB3. The photosynthetic bacteria used were isolated from seawater and identified as belonging to the genera *Rhodopseudomonas* and *Rhodobacter*. Meanwhile, the endophytic bacteria used were isolates coded BCB3, obtained from chili pepper seeds that were suspected to possess pathogen resistance.

Initial research stages encompassed the construction of experimental plots, seed selection, seedling cultivation, and transplanting. Plots measured 1.2 m in width and 10 m in length, with plant spacing of 60 cm x 70 cm. Organic fertilizer was applied at a rate of 20 tons/ha, supplemented with 200 kg/ha TSP. Concurrently, seed selection was performed. Seeds that submerged in warm water were selected for cultivation in seedling trays for two weeks. Transplanting occurred in the afternoon. Routine plant care involved the application of NPK 16:16:16 fertilizer at a rate of 2 g/L until 14 days after transplanting (DAT), increasing to 4 g/L until 42 HSPT, and finally 8 g/L until harvest, accompanied by weed control.

2.2 Propagation of Photosynthetic and Endophytic Bacteria, Isolation, and Antagonistic Testing

Rhodobacter sp. and *Rhodopseudomonas* sp. isolates were cultured in NC medium supplemented with 2 g/L sodium chloride for four days (Figure 1C). Subsequently, these cultures were inoculated into a nutrient-rich liquid medium and exposed to light for five days to stimulate growth (Figure 1A). The bacterial suspensions were gradually expanded to a 20-liter volume and maintained under light conditions until a characteristic reddish pigmentation developed (Figure 1B).

Endophytic bacteria were isolated from chili pepper plants exhibiting a high incidence of disease. Seed tissues were aseptically processed to recover endophytes, which were subsequently cultured on nutrient broth. Isolates were screened for antagonistic activity against *Colletotrichum gloeosporoides*, *Fusarium oxysporum*, and *Ralstonia solanacearum* using a dual culture assay. Isolate BCB3, demonstrating broad-spectrum inhibition, was selected for further study. Isolate BCB3 underwent aseptic subculturing to generate sufficient biomass (Figure 2A). An initial 5 ml culture was transferred to a 50 ml Erlenmeyer flask containing

nutrient broth and incubated on a rotary shaker for three days prior to application (Figure 2B).

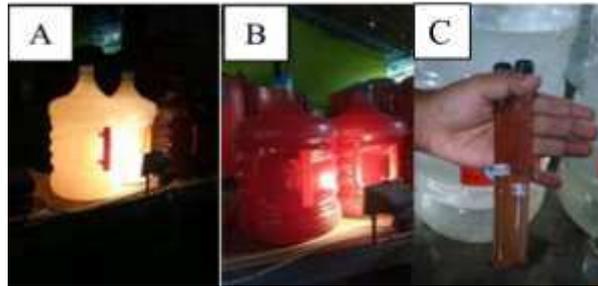


Figure 1. Isolation and Propagation of Photosynthetic Bacteria

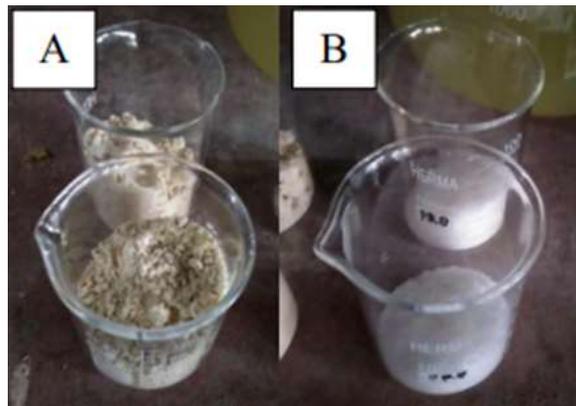


Figure 2. Isolate of Endophytic Bacteria (A) and Bacterial Solution (B)

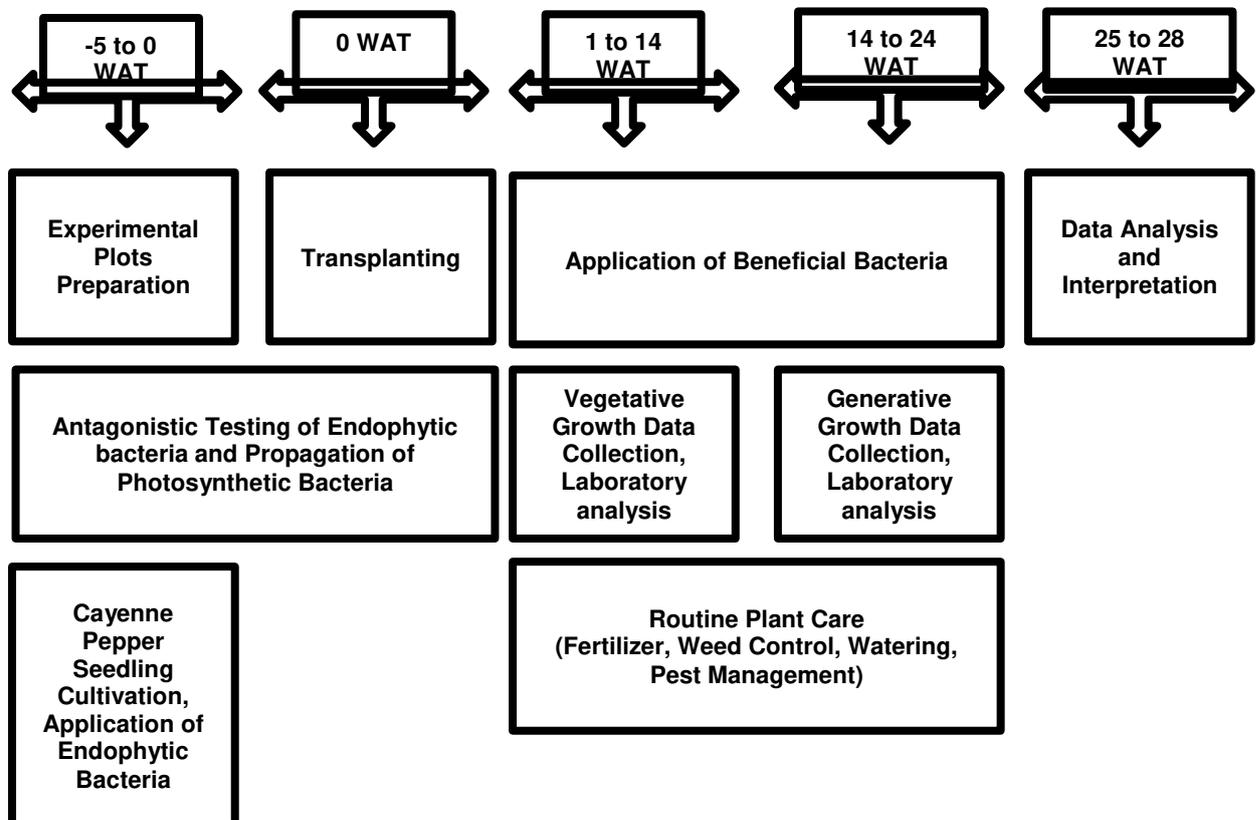


Figure 3. Research Implementation Process

2.3 Research Design and Data Analysis

A randomized complete block design (RCBD) was employed to evaluate the effects of different bacterial treatments. The experiment consisted of four treatments: (P1) application of both photosynthetic and endophytic bacteria, (P2) application of photosynthetic bacteria, (P3) application of endophytic bacteria, and (P4) control (no bacterial application). Each treatment was replicated ten times, with four plots per replication, resulting in a total of 40 experimental units. Plots were separated by a distance of two meters.

Photosynthetic bacteria were applied weekly at a rate of 10 mL/L, while endophytic bacteria were applied at a rate of 5 mL/L. Both of these bacteria were applied using foliar application technique. Seeds for treatments P1 and P3 underwent a 1-hour pre-sowing soak in a 5 mL/L endophytic bacterial solution. The soaked seeds were then drained and sown. Figure 3 provides an overview of the research implementation process. Plant height, leaf area, dichotomous branch height, chlorophyll content, flower number, fruit number per plant, fresh fruit weight, fruit length, fruit diameter, yield, and disease incidence (anthracnose, bacterial wilt, and Fusarium wilt) were recorded. Data were subjected to analysis of variance (ANOVA) to determine treatment effects. Tukey's Honestly Significant Difference (HSD) test was used for pairwise comparisons among treatment means when significant differences were detected. Statistical analyses were performed using the agricolae package in R-4.3.1.

3. RESULT AND DISCUSSION

The combined application of photosynthetic and endophytic bacteria exhibited differential effects on chili pepper plant growth. Figure 4A illustrates that both combined and individual treatments significantly enhanced plant

height up to 14 WAT. However, bacterial treatments did not influence dichotomous branch height development (Figure 4B). Photosynthetic bacteria potentially augmented plant photosynthesis, thereby stimulating overall growth (Xu et al., 2016). Meanwhile, endophytic bacteria can enhance nutrient availability for plants. Endophytes have been reported to fix atmospheric nitrogen using the enzyme nitrogenase and convert it into a plant-available form (Ivleva et al., 2016). Furthermore, endophytic bacteria can produce plant hormones such as auxins and gibberellins, which can stimulate plant growth (Shi et al., 2014).

Based on the chlorophyll content in leaves (Figure 4C), there was a difference between single and combined bacterial treatments. Chili pepper plants treated with PSB alone and in combination with endophytes had similar chlorophyll A and B contents but differed significantly from the single endophyte and control treatments. The combined application of photosynthetic bacteria and endophytes resulted in a stronger synergistic effect in increasing chlorophyll production in plants. These findings are in agreement with Yen et al. (2022) who studied rice plants. Plants treated with PSB exhibited increased chlorophyll content due to the synthesis of ALA by the bacteria. 5-Aminolevulinic acid is a primary precursor for chlorophyll (Wu et al., 2019). Enhanced chlorophyll accumulation is likely attributable to elevated ALA biosynthesis within the soil microenvironment facilitated by heightened PSB activity. Yuan et al. (2018) reported that endophytic bacteria can enhance chlorophyll content in *P. edulis* plants by stimulating the biosynthesis of 5-aminolevulinic acid (ALA). The morphological characteristics of chili pepper plants under various beneficial bacterial treatments are presented in Figure 5.

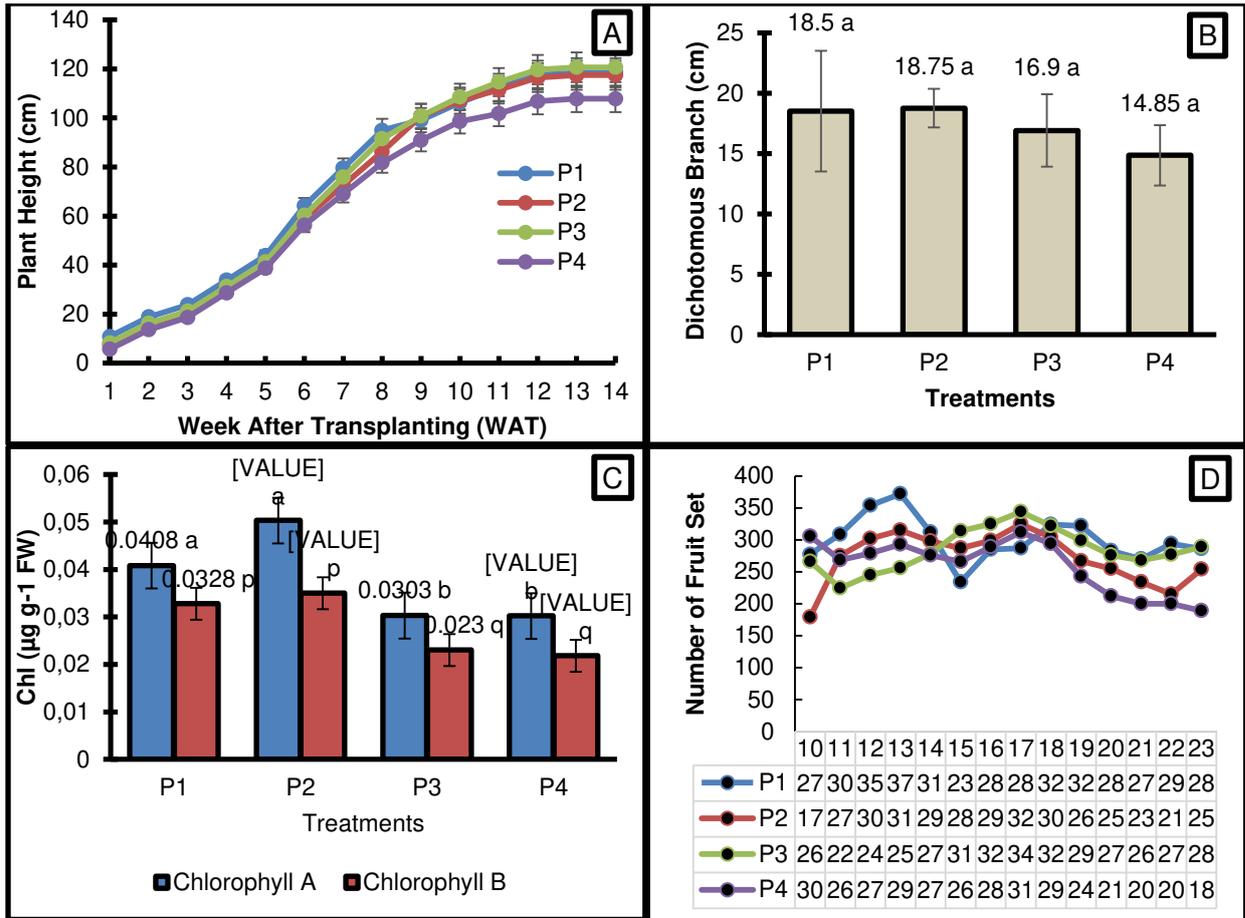


Figure 4. Growth Parameter of Cayenne Pepper, Note: P1 (PSB + Endophyte), P2 (PSB Only), P3 (Endophyte Only), P4 (Control).



Figure 5. Morphological features of chili plants at 42 DAT, Note: P1 (PSB + Endophyte), P2 (PSB Only), P3 (Endophyte Only), P4 (Control).

In the variable of flower and bud count, observed from 10 WAT onwards, a significant decrease was observed in the combined treatment group at the 15th week (Figure 4D). This phenomenon might be attributed to the cumulative effects of bacterial application over this period, where plants may have received

sufficient nutrients and support to produce flowers optimally. However, after the 15th week, both flower and bud counts increased until the final observation at the 23rd week. In the final week of observation, the combined PSB + endophyte and endophyte-only treatments yielded the highest flower

counts compared to other treatments. Similar findings were reported by Reut et al. (2024), who demonstrated that the application of the endophytic bacterium *Bacillus subtilis* accelerated flowering and increased flower number in lily plants. Additionally, the photosynthetic bacterium *Rhodospseudomonas palustris* was

reported to enhance flower production in tomato plants. Furthermore, flowers produced under *R. palustris* treatment exhibited accelerated development compared to control plants without bacterial application (S.-K. Lee et al., 2022).

Table 1. Parameters of cayenne pepper yield components

Parameters	PSB + Endophyte	PSB	Endophyte	Control
Leaf Area (cm ²)	531.41 ± 2.15 a	515.44 ± 2.05 a	486.77 ± 3.10 a	489.12 ± 2.87 a
Fruit Number per Plant	725.14 ± 8.12 a	696.69 ± 7.55 a	654.32 ± 6.90 b	658.22 ± 7.02 b
Fruit Fresh Weight (g)	1.2 ± 0.08 a	0.99 ± 0.10 ab	0.81 ± 0.12 b	0.79 ± 0.11 b
Fruit Length (cm)	5.12 ± 0.22 a	5.09 ± 0.25 a	4.88 ± 0.30 a	4.45 ± 0.32 b
Fruit Diameter (cm)	1.10 ± 0.04 a	1.08 ± 0.05 a	1.08 ± 0.06 a	1.02 ± 0.07 a
Plant Production (kg)	0.87 ± 0.03 a	0.69 ± 0.05 ab	0.53 ± 0.07 b	0.52 ± 0.06 b
Productivity (ton ha ⁻¹)	18.71 ± 0.22 a	14.84 ± 0.31 a	11.39 ± 0.42 b	11.18 ± 0.40 b

Note: The values shown are the means followed by a notation. The same letters indicate no significant difference based on Tukey's HSD test at $\alpha = 5\%$.

Table 1 presents the yield component parameters of chili pepper plants subjected to various beneficial bacterial treatments. No significant differences in leaf area were observed among treatments. This finding deviates from previous research indicating that the application of endophytic or photosynthetic bacteria can enhance leaf area expansion (Akköprü et al., 2021; Andrade et al., 2023). Meanwhile, the number of fruits per plant was significantly influenced by beneficial bacteria, with the combined PSB + endophyte and PSB-only treatments showing significant differences compared to the endophyte-only and control treatments (Table 1). Treatment P1 exhibited the highest average fruit number per plant (725.14 ± 8.12), followed by P2 (696.69 ± 7.55), while P3 and P4 showed lower averages (654.32 ± 6.90 and 658.22 ± 7.02, respectively). These results suggest that photosynthetic bacteria had a significant impact on fruit number, whereas endophytes had no discernible effect. A similar pattern was observed for fresh fruit weight. The combined beneficial bacterial treatment differed significantly from the other treatments and yielded the highest fruit

weight. Endophytes did not exhibit any effect, as their fruit weight values were similar to the control. In terms of fruit size, fruit diameter was unaffected by beneficial bacteria (Table 1). Conversely, the application of all beneficial bacteria significantly increased fruit length compared to the control (Figure 6). Azmi et al. (2022) reported that the combination of endophytic bacteria and seaweed extract could influence the diameter and length of *Capsicum annuum*.

Fruit yield per plant, defined as the total number of fruits produced by an individual chili pepper plant, was significantly higher in plants treated with the combined PSB+endophyte or PSB alone compared to other treatments (Table 1). Consequently, these treatments also resulted in the highest productivity per hectare. The application of photosynthetic bacteria led to a 32.69% increase in productivity, while the combination of PSB and endophytes resulted in an even greater increase of 67.31%. These findings align with those of K.-H. Lee et al. (2008), who observed increased fruit yield and weight in tomatoes following the application of photosynthetic bacteria

Rhodopseudomonas sp. KL9. Similarly, Yoon et al. (2012) reported an increase in the number and weight of chili peppers after treatment with *Rhodopseudomonas capsulatus*. Conversely, Díaz-Pérez (2022) reported contrasting results, finding no significant improvement in growth or yield in pepper plants treated with photosynthetic bacteria. Endophytic

bacteria have also been shown to enhance chili pepper yield (Purwanti et al., 2021). The combined application of cattle manure and endophytic bacteria every five days significantly increased fruit number per plant, leading to a productivity increase of up to 19.5 tons per hectare.



Figure 6. Morphological characteristics of chili fruits as influenced by different beneficial bacterial treatments, Note: P1 (PSB + Endophyte), P2 (PSB Only), P3 (Endophyte Only), P4 (Control).

Cayenne pepper farmers often encounter numerous challenges, including plant diseases caused by various pathogens. Table 2 reveals that the severity of *Cercospora capsicii* leaf spot could be mitigated by the application of PSB alone, endophytes alone, or a combination of both. However, while endophytes alone suppressed *C. capsicii* development by $18.79 \pm 1.20\%$, the combination of endophytes and PSB only reduced it by $25.04 \pm 1.65\%$, indicating that endophytes were more effective on their own. For anthracnose, caused by *Colletotrichum gloeosporioides*, a slightly different pattern emerged. PSB treatment was less effective in suppressing this pathogen, with an average reduction of $13.20 \pm 0.90\%$, which was not significantly different from the control ($18.20 \pm 1.10\%$). In contrast, both endophyte-alone and combined PSB+endophyte treatments significantly reduced the incidence of anthracnose to $5.43 \pm 0.35\%$ and $4.19 \pm 0.25\%$, respectively. For Fusarium wilt caused by *Fusarium oxysporum*, no significant effect was observed from the application of

beneficial bacteria. The combined treatment of Photosynthetic Bacteria and Endophytes (P1) is the most effective in controlling the incidence of *Ralstonia solanacearum* in cayenne pepper, with no detectable presence of the pathogen. This indicates a synergistic effect, where the combination of these treatments significantly enhances the plant's defense mechanisms. In contrast, the use of Photosynthetic Bacteria alone (P2) resulted in a moderate incidence of *R. solanacearum* ($4.87 \pm 0.45b$), indicating that while beneficial, this treatment is less effective without the addition of Endophytes. The Endophyte-only treatment (P3) also provided some level of protection, with a lower incidence ($1.28 \pm 0.20a$), suggesting that Endophytes alone can inhibit the pathogen but not as robustly as the combined treatment. The untreated plants (P4) showed the highest incidence of *R. solanacearum* ($5.99 \pm 0.55b$), highlighting the pathogen's ability to proliferate in the absence of any bacterial intervention. Based on these results, endophytic bacteria demonstrated a more pronounced ability

to suppress pathogen growth in chili pepper plants compared to photosynthetic bacteria.

Table 2. Severity of disease infestation in cayenne pepper plants

Treatments	<i>C. capsicii</i>	<i>C. gloeosporoides</i>	<i>F. oxysporum</i>	<i>R. solanacearum</i>
PSB + Endophyte	25.04 ± 1.65 a	4.19 ± 0.25 a	0 ± 0.00 a	0 ± 0.00 a
PSB	31.78 ± 2.10 ab	13.20 ± 0.90 b	0 ± 0.00 a	4.87 ± 0.45 b
Endophyte	18.79 ± 1.20 a	5.43 ± 0.35 a	0 ± 0.00 a	1.28 ± 0.20 a
Control	54.32 ± 3.15 b	18.20 ± 1.10 b	0 ± 0.00 a	5.99 ± 0.55 b

Note: The values shown are the means followed by a notation. The same letters indicate no significant difference based on Tukey's HSD test at $\alpha = 5\%$.

Adedire et al. (2019) demonstrated that seed treatment with *Lactobacillus plantarum* effectively suppressed the growth of *Cercospora capsici*. Similarly, Yanti et al. (2019) reported that a consortium of *Bacillus* endophytes (*Bacillus pseudomycooides* strain SLBE 3.1 AP, *Bacillus thuringiensis* strain SLBE 2.3 BB, *Bacillus toyonensis* strain AGBE 2.1 TL) effectively controlled anthracnose disease. Nurbailis et al. (2023) also identified another endophyte strain capable of suppressing anthracnose. The most effective consortium was a combination of *Bacillus cereus* SNE 2.2 and *Bacillus cereus* TLE 1.1, which completely suppressed anthracnose, increased plant height by 58.33 cm, and increased fruit weight by 208.97 grams.

Sundaramoorthy et al. (2011), demonstrated that a combination of PGPR and endophytic bacteria effectively controlled *Fusarium* wilt in chili peppers under both greenhouse and field conditions. This efficacy was attributed to the induction of systemic resistance (ISR), which enhanced the activity of plant defense enzymes. The increased enzyme activity triggered the synthesis of phytoalexins, antifungal compounds that suppressed *Fusarium* growth. Yanti et al. (2018) identified nine endophytic bacterial isolates capable of controlling both bacterial and *Fusarium* wilts in chili peppers. Six isolates suppressed bacterial wilt without causing symptoms, while five others suppressed *Fusarium* wilt symptoms. Three isolates, namely *Bacillus pseudomycooides* strain NBRC 101232, *Bacillus thuringiensis* strain

ATCC 10792, and *Bacillus mycooides* strain 273, showed the most promising potential for controlling both wilts in chili peppers.

In general, endophytic bacteria can enhance plant growth by providing nutrients, improving water and mineral uptake, or producing plant growth regulators. Healthy and vigorous plants are more resistant to disease (Singh et al., 2020). Endophytic bacteria can also induce plant defense systems by increasing the production of plant defense hormones, triggering hypersensitive responses, or strengthening plant cell walls. This renders plants more resistant to pathogen attacks (Podolich et al., 2015).

4. CONCLUSION

The application of photosynthetic bacteria (PSB) and endophytic bacteria, both individually and in combination, can enhance growth and yield of chili pepper plants at the field scale. Combined treatments resulted in increased plant height, chlorophyll content, flower number, fruit number per plant, fresh fruit weight, yield, productivity, and resistance to *C. capsici*, *C. gloeosporoides*, *F. oxysporum*, and *R. solanacearum*. Sole application of PSB improved plant growth attributes, while endophytes showed potential in enhancing chili pepper resistance to pathogens.

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