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Vegetative Growth of Maize Following *Bacillus Thuringiensis* Suspension Application**Christina Leta Salaki^{1*}, Henny Vianne Gretha Makal¹, Yefta Pamandungan²**¹Plant Protection Study Program, Faculty of Agriculture, Sam Ratulangi University²Agronomy Study Program, Faculty of Agriculture, Sam Ratulangi University*Corresponding author:
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Abstract. Microbial inoculants are being evaluated as complementary options for supporting maize (*Zea mays* L.) growth, but responses to different application volumes of *Bacillus thuringiensis* remain insufficiently characterized under pot cultivation. This study assessed maize vegetative growth following application of a bacterial suspension. The experiment was conducted at the Faculty of Agriculture, Sam Ratulangi University, Manado, Indonesia, from May to August 2026. Six treatment levels were reported: a control and five bacterial suspension treatments (P1–P5), corresponding to nominal volumes of 1, 3, 5, 7, and 9 mL of suspension per 10 mL of water, respectively, with four replicates per treatment. Plant height and recorded leaf number were used as vegetative growth indicators. Mean plant height ranged from 192.3 cm in the control to 246.3 cm in P5, a difference of 54.0 cm (28.1%). P4 reached 238.5 cm, whereas P1–P3 ranged from 193.3 to 201.8 cm. Recorded leaf number ranged from 3.0 in the control to 4.0 under P3–P5. Because the underlying analysis-of-variance results and measures of dispersion were not available for verification, treatment differences were interpreted descriptively rather than as statistically significant effects. The observed pattern suggests an association between bacterial suspension treatment and maize vegetative characteristics. Clarification of experimental records and further evaluation are needed before recommending an application level or inferring effects on soil fertility, pest suppression, or grain yield.

Keywords: Bacterial inoculant; Biofertilizer; Leaf number; Plant height; *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop for food, animal feed, and agricultural industries. Improving maize growth remains a central challenge in production systems where nutrient management and soil conditions limit plant development. In addition to conventional fertilization, the use of beneficial microorganisms has attracted attention as a potential approach for supporting crop growth. In maize, rhizobacterial inoculation has produced favorable growth responses under certain soil and management conditions (Amogou et al., 2021; Ribeiro et al., 2022). For

example, Agunbiade et al. (2024) characterized drought-tolerant, plant-growth-promoting rhizobacteria relevant to maize productivity, further illustrating the diversity of candidate microbial inoculants.

Microbial inoculants may influence plant development through diverse interactions with the rhizosphere and roots. Some plant growth-promoting rhizobacteria can mobilize nutrients, produce growth-related metabolites, or modify the functioning of the root environment (Pirttilä et al., 2021; Shen et al., 2021). The performance of inoculants, however, depends on the properties of the isolate, the host crop, and experimental



conditions. Thus, beneficial characteristics observed for one microbial strain should not automatically be attributed to other isolates of the same species. In a maize study conducted under low-nitrogen conditions, Foster et al. (2025) found that both inoculation frequency and host genotype influenced growth responses to bacterial isolates, highlighting the need to define application procedures accurately.

Members of the genus *Bacillus* are of particular interest in applied agricultural microbiology because they include spore-forming bacteria with a broad range of functional traits. Selected isolates may promote plant growth or support beneficial interactions in root-associated microbial communities (Shen et al., 2023; Tsotetsi et al., 2022). These effects are not universal, and their practical value requires evaluation for the isolate and application procedure used in each study. In wheat, Mirskaya et al. (2022) evaluated developmental responses to an inoculated hormone-producing *Bacillus* strain, illustrating that effects documented for one bacterium–crop combination should not be generalized to another.

Bacillus thuringiensis (Bt) is best known for its insecticidal proteins and applications in biological pest management. However, certain strains have also demonstrated plant growth-promoting properties. de Almeida et al. (2021) reported endophytic colonization of maize roots and leaves by *B. thuringiensis* strain RZ2MS9. Subsequently, Figueredo et al. (2023) demonstrated that indole-3-acetic acid biosynthesis contributed to the ability of this strain to promote maize growth. These findings suggest that the agricultural relevance of selected *B. thuringiensis* strains can extend beyond insect pest control. Ali et al. (2022) also reported that *B. thuringiensis* PM25 influenced maize growth and stress-related physiological traits under salinity; those stress-specific

findings should not be assumed to apply to plants grown without the same stress treatment.

Other investigations have explored microbial inoculation in maize under contrasting conditions. Co-inoculation with selected *Bacillus* and *Azospirillum* strains improved plant growth and nutrient uptake compared with some single-inoculation treatments (Ribeiro et al., 2022). Ferrarezi et al. (2022) also showed that inoculation with plant growth-promoting rhizobacteria can influence the maize-associated bacterial community in field conditions. More recently, Bini et al. (2026) evaluated *B. thuringiensis* strain CNPMS B116 as a bioinoculant in relation to phosphorus-use efficiency and maize grain yield. Collectively, this literature underscores both the potential of microbial inoculants and the importance of strain-specific, context-dependent assessment. In another maize experiment, Yassue et al. (2023) assessed growth-related traits following inoculation with a bacterial consortium under nitrogen stress, underscoring the relevance of crop genotype and experimental context.

Despite these advances, growth responses to particular application volumes of bacterial suspensions remain dependent on inoculum preparation and cultivation conditions. Simple measurements, such as plant height and leaf number, can provide a preliminary description of maize vegetative responses to microbial treatment. Such measurements cannot, on their own, establish changes in soil fertility, nutrient uptake, pest infestation, or grain production. An assessment that distinguishes observed growth responses from unmeasured mechanisms is therefore appropriate for initial evaluation of a bacterial inoculant.

Accordingly, this study aimed to compare maize vegetative growth under five nominal volumes of *B. thuringiensis* suspension and a control in a polybag cultivation experiment. Plant height and



recorded leaf number were evaluated to provide preliminary evidence on the association between bacterial suspension treatments and maize growth characteristics.

MATERIAL AND METHODS

Study Area and Duration

The experiment was conducted from May to August 2026 at the Entomology and Plant Pest Laboratory, Faculty of Agriculture, Sam Ratulangi University, Manado, North Sulawesi, Indonesia. Maize plants were grown in polybags, and vegetative responses to *Bacillus thuringiensis* suspension were recorded.

Biological Materials and Inoculum Preparation

The principal biological materials comprised a pure culture identified in the original study as *Bacillus thuringiensis* and maize plants. Nutrient agar (NA), water, cotton, polybags, and alcohol were listed among the materials. A loopful of bacterial culture was streaked onto fresh NA and incubated at room temperature for 24 h. The study record also describes collecting bacterial material with a cotton swab from an agar slant, suspending the biomass in 10 mL of water, and shaking the suspension for 30 min. The source and strain identity of the culture, as well as viable cell density, were not documented in the available record.

Experimental Treatments and Application

Six treatment levels were reported, comprising a control (P) and five volumes of bacterial suspension: P1, 1 mL; P2, 3 mL; P3, 5 mL; P4, 7 mL; and P5, 9 mL. These volumes were described in relation to 10 mL of water per plant. Each treatment was replicated four times. The reported volumes are retained as nominal treatment designations because the record does not establish whether 10 mL represented the diluent quantity or the

final application volume. The original documentation is also inconsistent regarding complete randomization versus randomized blocks, so a specific allocation design is not assigned in this revision.

The prepared formulation was poured onto the growing medium around the planting hole of maize plants in polybags. The available study description does not specify the first application date, application frequency, control watering procedure, maize cultivar, growth-medium composition, or other fertilizer inputs. Consequently, these conditions cannot be reconstructed from the manuscript.

Vegetative Measurements and Data Analysis

Plant height (cm) and recorded leaf number were the two response variables. The source methodology refers to observations during the vegetative period, approximately 2–7 weeks after planting; however, the original results narrative and table use conflicting day-after-application designations. For this reason, no unverified sampling date is assigned to the means reported below.

The original methods mention analysis of variance (ANOVA) followed by an unspecified post hoc procedure, but the ANOVA output, post hoc results, and measures of within-treatment variation were not available for confirmation. Accordingly, this manuscript summarizes the reported treatment means descriptively. Relative differences from the control were calculated as $((\text{treatment mean} - \text{control mean}) / \text{control mean}) \times 100$. No statistical significance, confidence intervals, or error bars were inferred from the available data.

RESULTS AND DISCUSSION

Results

Mean plant height and recorded leaf number for the control and five bacterial



suspension treatments are presented in Table 1. Both variables showed numerical differences among treatments, although their descriptive patterns were not identical.

Table 1. Mean maize plant height and leaf number by *Bacillus thuringiensis* treatment

Treatment	Plant height (cm)	Recorded leaf number
P (control)	192.3	3.0
P1 (1 mL)	193.3	3.5
P2 (3 mL)	195.5	3.5
P3 (5 mL)	201.8	4.0
P4 (7 mL)	238.5	4.0
P5 (9 mL)	246.3	4.0

The mean plant height increased from 192.3 cm in the control to 246.3 cm under P5. The absolute contrast between these two treatments was 54.0 cm (28.1% relative to the control). P4 reached 238.5 cm (24.0% above the control), whereas the

values under P1–P3 were comparatively close to the control, ranging from 193.3 to 201.8 cm. The largest adjacent increase occurred between P3 and P4 (36.7 cm). These differences are descriptive because the original inferential statistics have not been verified.

The recorded mean leaf number increased from 3.0 in the control to 3.5 under P1 and P2, reaching 4.0 under P3–P5. Thus, the leaf-number indicator reached the same reported mean in the three highest treatment levels even as mean plant height continued to increase. Confirmation of the original leaf-counting definition is important because the counts are low relative to the reported plant heights.

Figure 1 expresses the treatment contrasts as percentages relative to the corresponding control mean. This normalization highlights the different descriptive responses of the two vegetative indicators without implying a statistical test.

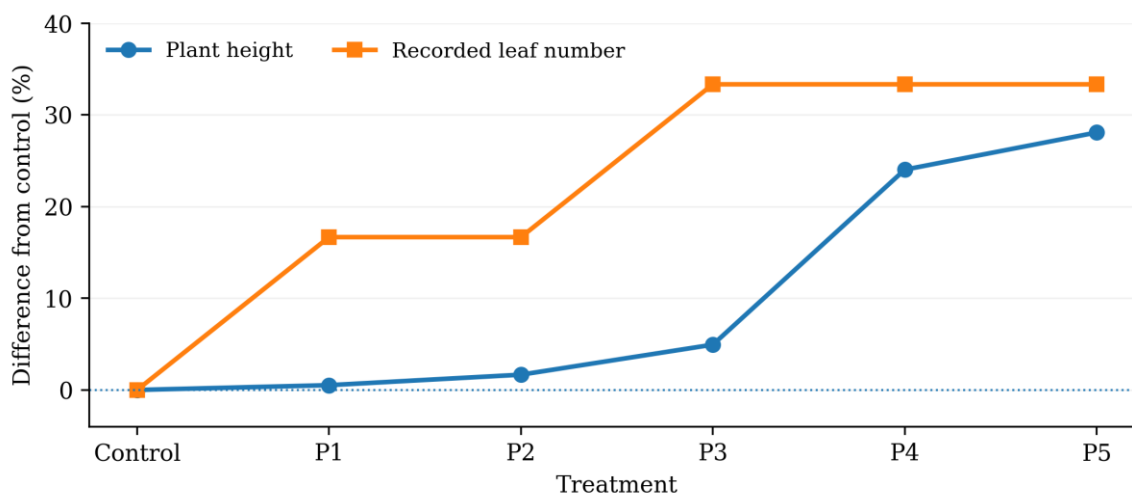


Figure 1. Relative difference (%) in mean plant height and recorded leaf number from the control across bacterial suspension treatments.

Discussion

The highest reported mean plant height occurred under the largest nominal suspension volume (P5). The overall pattern is compatible with the possibility that a *Bacillus thuringiensis* inoculant

influenced maize vegetative growth, but this interpretation remains preliminary. In particular, the data cannot establish whether the treatment differences were statistically significant or whether the

apparent response was due to a specific biological trait of the bacterial isolate.

Selected *B. thuringiensis* strains have exhibited plant growth-promoting activity in previous investigations. de Almeida et al. (2021) documented endophytic colonization of maize roots and leaves by strain RZ2MS9. Figueredo et al. (2023) subsequently showed that indole-3-acetic acid biosynthesis contributed to the growth-promoting activity of the same strain. These findings provide a mechanistic framework for interpreting responses to certain *B. thuringiensis* inoculants, but the strain used in the present experiment was not sufficiently characterized to establish the operation of the same pathway. Outside maize, Dal’Rio et al. (2024) reported enhanced growth of *Tropaeolum majus* following co-inoculation with endophytes that included *B. thuringiensis* CAPE95; this consortium-based result cannot be assigned to a single bacterial strain or transferred directly to the present maize experiment.

Microbial effects on plant nutrition represent another possible explanation. Phosphate-solubilizing bacteria can alter rhizosphere metabolite profiles, and inoculation with selected *Bacillus*-containing formulations has been associated with differences in maize growth and nutrient uptake (Shen et al., 2021; Ribeiro et al., 2022). In a separate maize study, Bini et al. (2026) reported improved phosphorus-use efficiency and grain yield following application of *B. thuringiensis* strain CNPMS B116. However, the present experiment did not measure root colonization, nutrient uptake, soil nutrient concentrations, hormone production, or microbial cell density; causal inferences about these processes are therefore unwarranted. Kaur et al. (2024) likewise reported improved growth-related responses in fodder maize inoculated with a chromium-tolerant *B. thuringiensis* isolate under chromium stress, but their findings concerned a

different bacterial strain and experimental stress context.

The different trajectories for height and leaf count indicate that the two reported indicators should be interpreted separately. While mean height rose most noticeably between P3 and P4, the reported leaf count did not increase beyond P3. Similarities with previous studies may reflect the broad range of beneficial interactions described for *Bacillus* spp., but also emphasize why observations for one trait cannot be used to infer responses of other growth or yield components (Tsotetsi et al., 2022; Shen et al., 2023).

From an applied agroecotechnology perspective, the study offers an initial comparison of maize vegetative responses to different nominal *B. thuringiensis* suspension volumes. Its practical interpretation remains limited by incomplete information on inoculum concentration, randomization, application frequency, and timing of assessment. The recorded increase in plant height should not be treated as evidence of an optimal application rate or as proof of improved soil fertility, reduced pest infestation, or higher grain yield. Confirmatory trials with documented inoculum density, experimental allocation, replicated measurements, and yield-related observations would allow more reliable assessment of agricultural utility (Ferrarezi et al., 2022; Pirttilä et al., 2021). Recent evidence that inoculation frequency can change maize growth responses (Foster et al., 2025) reinforces the importance of documenting application timing and repetition before making agronomic recommendations.

CONCLUSION

Under the reported experimental conditions, *Bacillus thuringiensis* suspension treatments were associated with numerical differences in maize vegetative characteristics. P5 (9 mL



nominal suspension volume) had the highest recorded mean plant height (246.3 cm), compared with 192.3 cm in the control. The highest recorded mean leaf number (4.0) was shared by P3, P4, and P5, compared with 3.0 in the control. These descriptive findings provide preliminary support for assessing *B. thuringiensis* as a candidate microbial growth-promoting agent. Verification of the experimental records and statistical outputs is needed before drawing inferential conclusions or recommending an agricultural application rate.

REFERENCES

- Agunbiade, V. F., Fadiji, A. E., Agbodjato, N. A., & Babalola, O. O. (2024). Isolation and characterization of plant-growth-promoting, drought-tolerant rhizobacteria for improved maize productivity. *Plants*, 13(10), Article 1298. <https://doi.org/10.3390/plants13101298>
- Ali, B., Hafeez, A., Ahmad, S., Javed, M. A., Sumaira, Afridi, M. S., Dawoud, T. M., Almaary, K. S., Muresan, C. C., Marc, R. A., Alkhalifah, D. H. M., & Selim, S. (2022). *Bacillus thuringiensis* PM25 ameliorates oxidative damage of salinity stress in maize via regulating growth, leaf pigments, antioxidant defense system, and stress responsive gene expression. *Frontiers in Plant Science*, 13, Article 921668. <https://doi.org/10.3389/fpls.2022.921668>
- Amogou, O., Noumavo, A. P., Agbodjato, N. A., Sina, H., Dagbénonbakin, G., Adoko, M. Y., Salako, V. K., Glèlè Kakaï, R., Adjanohoun, A., & Baba-Moussa, L. (2021). Rhizobacterial inoculation in combination with mineral fertilizer improves maize growth and yield in poor ferruginous soil in center of Benin. *BioTechnologia*, 102(2), 141–155. <https://doi.org/10.5114/bta.2021.106520>
- Bini, D., Ribeiro, V. P., Marins, M. S., Silva, F. C., Rodrigues, V. A., Santos, B. R., Gomes, E. A., dos Santos, F. C., de Paula Lana, U. G., Coelho, A. M., Marriel, I. E., de Sousa, S. M., & de Oliveira-Paiva, C. A. (2026). *Bacillus thuringiensis* CNPMS B116, a Brazilian strain from Cerrado, as a bioinoculant for improving maize yield and phosphorus use efficiency. *Archives of Microbiology*, 208(1), Article 14. <https://doi.org/10.1007/s00203-025-04564-0>
- Dal’Rio, I., Lopes, E. dos S., Santaren, K. C. F., Rosado, A. S., & Seldin, L. (2024). Co-inoculation of the endophytes *Bacillus thuringiensis* CAPE95 and *Paenibacillus polymyxa* CAPE238 promotes *Tropaeolum majus* L. growth and enhances its root bacterial diversity. *Frontiers in Microbiology*, 15, Article 1356891. <https://doi.org/10.3389/fmicb.2024.1356891>
- de Almeida, J. R., Bonatelli, M. L., Batista, B. D., Teixeira-Silva, N. S., Mondin, M., dos Santos, R. C., Bento, J. M. S., de Almeida Hayashibara, C. A., Azevedo, J. L., & Quecine, M. C. (2021). *Bacillus thuringiensis* RZ2MS9, a tropical plant growth-promoting rhizobacterium, colonizes maize endophytically and alters the plant’s production of volatile organic compounds during co-inoculation with *Azospirillum brasilense* Ab-V5. *Environmental Microbiology Reports*, 13(6), 812–821. <https://doi.org/10.1111/1758-2229.13004>
- Ferrarezi, J. A., Carvalho-Estrada, P. de A., Batista, B. D., Aniceto, R. M., Tschoeke, B. A. P., Andrade, P. A. de M., Lopes, B. de M., Bonatelli, M. L., Odisi, E. J., Azevedo, J. L., & Quecine, M. C. (2022). Effects of inoculation with plant growth-promoting rhizobacteria from the Brazilian Amazon on the bacterial community associated with maize in field. *Applied Soil Ecology*, 170, Article 104297. <https://doi.org/10.1016/j.apsoil.2021.104297>
- Figueroa, E. F., da Cruz, T. A., de Almeida, J. R., Batista, B. D., Marcon, J., de Andrade, P. A. M., de Almeida Hayashibara, C. A., Rosa, M. S.,



- Azevedo, J. L., & Quecine, M. C. (2023). The key role of indole-3-acetic acid biosynthesis by *Bacillus thuringiensis* RZ2MS9 in promoting maize growth revealed by the *ipdC* gene knockout mediated by the CRISPR-Cas9 system. *Microbiological Research*, 266, Article 127218. <https://doi.org/10.1016/j.micres.2022.127218>
- Foster, L. R., Yang, J., Riethoven, J.-J. M., Mukhtar, H., & Schachtman, D. P. (2025). Inoculation frequency and maize genotype influence plant growth-promoting effects of soil bacteria under low nitrogen conditions. *Frontiers in Plant Science*, 16, Article 1637156. <https://doi.org/10.3389/fpls.2025.1637156>
- Kaur, H., Katyal, P., Chandel, S., Singh, D., Kumar, P., & Choudhary, M. (2024). Microbes mediated alleviation of chromium (Cr VI) stress for improved phytoextraction in fodder maize (*Zea mays* L.) cultivar. *Heliyon*, 10(23), Article e40361. <https://doi.org/10.1016/j.heliyon.2024.e40361>
- Mirskaya, G. V., Khomyakov, Y. V., Rushina, N. A., Vertebny, V. E., Chizhevskaya, E. P., Chebotar, V. K., Chesnokov, Y. V., & Pishchik, V. N. (2022). Plant development of early-maturing spring wheat (*Triticum aestivum* L.) under inoculation with *Bacillus* sp. V2026. *Plants*, 11(14), Article 1817. <https://doi.org/10.3390/plants11141817>
- Pirttilä, A. M., Mohammad Parast Tabas, H., Baruah, N., & Koskimäki, J. J. (2021). Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. *Microorganisms*, 9(4), Article 817. <https://doi.org/10.3390/microorganisms9040817>
- Ribeiro, V. P., Gomes, E. A., de Sousa, S. M., de Paula Lana, U. G., Coelho, A. M., Marriel, I. E., & de Oliveira-Paiva, C. A. (2022). Co-inoculation with tropical strains of *Azospirillum* and *Bacillus* is more efficient than single inoculation for improving plant growth and nutrient uptake in maize. *Archives of Microbiology*, 204(2), Article 143. <https://doi.org/10.1007/s00203-022-02759-3>
- Shen, M., Li, J., Dong, Y., Liu, H., Peng, J., Hu, Y., & Sun, Y. (2021). Profiling of plant growth-promoting metabolites by phosphate-solubilizing bacteria in maize rhizosphere. *Plants*, 10(6), Article 1071. <https://doi.org/10.3390/plants10061071>
- Shen, Y., Yang, H., Lin, Z., Chu, L., Pan, X., Wang, Y., Liu, W., Jin, P., & Miao, W. (2023). Screening of compound-formulated *Bacillus* and its effect on plant growth promotion. *Frontiers in Plant Science*, 14, Article 1174583. <https://doi.org/10.3389/fpls.2023.1174583>
- Tsotetsi, T., Nepthali, L., Malebe, M., & Tugizimana, F. (2022). *Bacillus* for plant growth promotion and stress resilience: What have we learned? *Plants*, 11(19), Article 2482. <https://doi.org/10.3390/plants11192482>
- Yassue, R. M., Galli, G., Fritsche-Neto, R., & Morota, G. (2023). Classification of plant growth-promoting bacteria inoculation status and prediction of growth-related traits in tropical maize using hyperspectral image and genomic data. *Crop Science*, 63(1), 88–100. <https://doi.org/10.1002/csc2.20836>

