# Enhancing Tomato (*Lycopersicon esculentum* Mill.) Growth in a Greenhouse Using NPK Fertilizer Coated with Endospore-Forming *Bacillus*

**Reginawanti Hindersah[1]*, Betty Natalie Fitriatin[1], Mieke Rochimi Setiawati[1], Pujawati Suryatmana1, Rara Rahmatika Risanti[2],** dan **Yeni Wispa Dewi[2]**

[1]Departemen Ilmu Tanah dan Sumberdaya Lahan, Fakultas Pertanian, Universitas Padjadjaran
Jl. Ir. Soekarno km. 21, Jatinangor, 45363 Sumedang
[2]Laboratorium Biologi Tanah, Fakultas Pertanian, Universitas Padjadjaran
Jl. Ir. Soekarno KM 21, Jatinangor, 45363 Sumedang
*Alamat korespondensi: reginawanti@unpad.ac.id

**INFO ARTIKEL** | **ABSTRACT/ABSTRAK**

Diterima: 19-03-2025
Direvisi: 12-05-2025
Dipublikasi: 31-05-2025

Keywords:
Andisol, Biomassa, Media pertumbuhan, Populasi mikroba

**Peningkatan pertumbuhan tanaman tomat (*Lycopersicon esculentum* Mill.) di rumah kaca dengan pupuk NPK berlapis *Bacillus* pembentuk endospora**

Penggunaan pupuk komposit NPK dalam jangka panjang disertai pupuk hayati termasuk rizobakteri *Bacillus* pembentuk spora dapat memperbaiki kualitas dan pertumbuhan tanaman. Penelitian ini bertujuan untuk menganalisis pertumbuhan sel dan produksi endospora *Bacillus* pada tiga media cair inorganik dan mengobservasi efek dua formula NPK bersalut *Bacillus* (NBB) terhadap pertumbuhan tanaman tomat dalam pot dan populasi *Bacillus* di rizosfir. Evaluasi pertumbuhan *Bacillus* dilakukan di laboratorium sementara pengaruh formula NPK bersalut *Bacillus* terhadap pertumbuhan tanaman dilakukan dalam polibeg menggunakan Rancangan Acak Kelompok dengan perlakuan NPK 16-16-16 dosis rekomendasi, pupuk hayati cair konsorsium *Bacillus*, NBB formula C dan G dengan dosis masing-masing 100%, 75% dan 50% dari dosis rekomendasi, serta tanpa pupuk. Percobaan dilakukan menggunakan tanah Andisol. Hasil penelitian menunjukkan bahwa pada media Yuniarti, konsorsium *Bacillus* dapat menghasilkan populasi sel vegetatif dan spora tertinggi. Aplikasi NBB secara signifikan meningkatkan tinggi tanaman, jumlah daun, panjang akar, dan biomassa tomat dibandingkan kontrol sampai 6 minggu setelah tanam. Aplikasi 75% dosis rekomendasi NBB formula C dan G menghasilkan pertumbuhan tanaman terbaik. Populasi sel vegetatif dan endospora *Bacillus* di tanah tidak dipengaruhi oleh pemberian NBB, inokulan cair *Bacillus* maupun NPK konvensional. Penelitian ini mengindikasikan bahwa NBB berpotensi dapat menggantikan pupuk NPK 16-16-16 untuk tanaman tomat pada ordo tanah Andisol.

Kata Kunci:
Andisol, Biomass, Growth media, Microbial population

Long-term use of NPK composite fertilizers mixed with biofertilizers, including spore-forming *Bacillus* rhizobacteria, can improve plant quality and growth. This study aims to analyze *Bacillus's* growth and spore production in chemical-liquid growing media and to observe the effects of *Bacillus*-coated NPK formulas (BCN) on the growth of tomatoes in potted Andisol and the *Bacillus* population in the rhizosphere. The evaluation of *Bacillus* growth was conducted in the laboratory whereas the effect of the BCN was conducted as a pot experiment in a Randomized Block Design. The treatments included recommended dosage of NPK 16-16-16, liquid inoculant of *Bacillus* consortium, BCN-C, and BCN-G formulas with 100%, 75%, and 50% of the recommended dosage, respectively. Control plants were not treated with fertilizer. Laboratory assay verified that Yuniarti's medium supported *Bacillus*-consortium growth and produced the highest *Bacillus* vegetative cells and spores. The BCN application significantly increased plant height, number of leaves, root length, and tomato biomass compared to the control up to 6 weeks after planting. Three-quarters of the dosage of BCN-C and BCN-G gave the best growth of tomatoes. Introducing BCN and conventional NPK did not change the population of Bacillus vegetative and spore form in the rhizosphere. Results indicate that BCN can replace conventional NPK 16-16-16 fertilizer for tomato plants in Andisol soil order.

## INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are a horticultural commodity with high economic value and great demand in local and international markets. They are rich in nutrients, including vitamins C and K, potassium, and folate (Singh *et al.*, 2023). The best tomato growth requires a temperature between 18 and 28 °C during the growth phase and 14–24 °C in the reproductive phase (Ghaffarpour *et al.*, 2024). In tropical regions, tomatoes are best grown in the high altitudes of mountainous areas. However, volcanic soil Andisol dominates higher altitudes. Andisol soil is considered fertile soil and have good physical condition. Despite supporting vegetable production, such as tomatoes, the soil contained amorphous minerals of allophane composed of aluminum and silica (Yuliani *et al.*, 2017). Allophane has a high phosphate adsorption rate through a ligand-exchange mechanism that limits the P availability in soil (Airlangga *et al.*, 2020).

The NPK compound fertilizer has played a critical role in providing nutrients for horticultural production in Andisols. Overusing this chemical fertilizer raises the 2risks of soil degradation, decreases crop productivity, and causes water eutrophication (Chandini *et al.*, 2019; Hossain *et al.*, 2022). This challenge enhances the interest in biofertilizers that provide nutrients and phytohormones to enable plants to grow better.

*Bacillus* genera are the best candidates for biofertilizers, which have an inactive stage, forming the endospore resistant to desiccation and chemicals (Etesami *et al.*, 2023). The bacteria able to synthesize organic acid which can solubilize the unavailable inorganic phosphate to available one (Saeid *et al.*, 2018), and to produce phosphatase to degrade the organic phosphor (P) to soluble P (Fitriatin *et al.*, 2022). Specific *Bacillus* species, including *B. altitudinis* and *B. ssubtilis,*have been reported to provide P inorganic (Yue *et al.*, 2023; Jensen *et al.*, 2024), therefore, they are potentially useful for alleviating P deficiency.

Due to the presence and activity of the nifH gene, the wide range of *Bacillus* can fix inert $N_2$ to ammonia (Singh *et al.*, 2020) that is chemically changed to ammonium and enzymatically to nitrate (Hayatsu & Tago, 2021) for roots uptake. Moreover, *Bacillus* provides the phytohormones of auxin, cytokinins, and gibberellins (Poveda & González-Andrés, 2021) to promote plant growth. *Bacillus* species used in this study are reported to have multiple mechanisms as plant growth-promoting rhizobacteria (Risanti *et al.*, 2025)

Farmers are accustomed to applying NPK fertilizer during tomato production, but only a few have ever inoculated their plants with biofertilizers. Despite the importance of endospores in adapting to drought conditions in most parts of Indonesian dry land, a study on the sporulation of the *Bacillus* consortium in broth is limited. The formulation of *Bacillus*-based biofertilizer to increase the endospore population is required. Minerals, including calcium, magnesium, and manganese, can improve sporulation (Mirel *et al.*, 2010; Yuniarti *et al.*, 2019).

The effect of *Bacillus* on plant growth has been reported. Inoculating broccoli plants with *Bacillus* consortium liquid inoculant increased the plant height by 58% compared to the control (Salsabila *et al.*, 2024). A single strain of *B. velezensis* 83 improved the production of first-harvested tomatoes while *B. subtilis* QST713 promotes cauliflower growth (Balderas-Ruíz *et al.*, 2021; Suwarto & Muhammad, 2023). The application of *B. subtilis* MBI600 on tomato plants induces biochemical and molecular mechanisms and, hence, systemic resistance (Samaras *et al.*, 2021).

The separate application of bio- and chemical fertilizers could increase labor and production costs and become a challenge in fertilizer management. Coating NPK fertilizers with the *Bacillus*-based biofertilizer is an alternative approach for delivering the beneficial microbes directly to the rhizosphere simultaneously with NPK fertilizer. Microbial-coated fertilizers also contribute to more efficient nutrient utilization, potentially minimizing fertilizer inputs (Hindersah *et al.*, 2024). Generally, *Bacillus*-based biofertilizer formulation does not consider the sporulation capacity to withstand harsh conditions. The purposes of this study were to determine the *Bacillus* vegetative form and endospore count in specific growth media and to observe the growth response of tomatoes and changes in microbial populations in the rhizosphere after introducing various doses and types of *Bacillus*-coated NPK.

## MATERIAL AND METHODS

The research was conducted from May to October 2023 and comprised 1) a *Bacillus* consortium growth test in three endospore-inducing media and 2) a Greenhouse experiment to test two *Bacillus*-coated NPK fertilizer formulas. *B. safensis* B1, *B. subtilis* D2, *B. altitudinis* B14, and *Bacillus* sp. E2 was isolated from the vegetable's rhizosphere and identified through collaborative research between PT Pupuk Indonesia and the Faculty of Agriculture Universitas Padjadjaran (Unpad). The pot experiment was conducted at the greenhouse of the Faculty of Agriculture at 797 m above sea level.

### *Bacillus* viability test in liquid culture

The three tested mediums were *Bacillus* media, Yuniarti's media and modified Miler's media. *Bacillus* media composed of peptone 10 g, lactose 5 g, NaCl 5 g, beef extract 3 g, $K_2HPO_4$ 2 g, 1 L distilled water (Atlas, 2010). Whereas, Yuniarti's media consisted of Nutrient Broth (peptone 5 g and meat extract 3 g) mixed with KCl 1 g, $MgSO_4$ 0.25 g, $FeSO_4$ 1 mM, $CaCl_2$ 1 M and $MnCl_2.4H_2O$ 10 mM, 1 l distilled water (Yuniarti *et al.*, 2019): and Modified Mirel's media composed of nutrient broth 6 g, $MgSO4.7H_2O$, 0.5 g and KCl 2 g, 1 L distilled water (Mirel *at al.*, 2000).

Each bacterial species was proliferated in tryptic soy broth (peptone from casein 17.0 g, soy peptone 3.0 g, D (+) glucose monohydrate 2.5 g, sodium chloride 5.0 g, di-potassium hydrogen phosphate 2.5 g, distilled water 1 l) for 72 h at room temperature with 115 rpm agitation. One milliliter of each bacterial liquid inoculant was inoculated into 100 ml of each tested medium and incubated at room temperature on a 115-rpm gyratory shaker for 9 days. The vegetative and endospore populations were counted on days 3, 6, and 9 using a serial dilution plate method with tryptic soy agar (TSA) at 30 $^0$C. The last diluted bacterial suspension was heated to 80 $^0$C for 20 minutes to count the endospores. The average data from two replications were presented in the histogram.

### Pot Experiment

The experiment was set up in a Completely Randomized Block Design with eight treatments (Table 1) and five replications. The level of *Bacillus*-coated NPK (BCN) was based on the recommended dose of NPK 16-16-16 for tomatoes (1000 kg/ha) according to Subhan *et al.* (2009). Fertilizer dosages were calculated based on the substrate weight in individual pots (450 g), assuming that the density of Andisols is less than 0.9 g/cm$^3$ (Soil Survey Staff, 2014). Thus, the topsoil (20 cm) weighs 1,800 tons per hectare of land. Meanwhile, the dosage of *Bacillus* liquid inoculant was 5 l/ha, equal to 0.5 ml/plant. The BCN-C and BCN-G are differentiated based on the zeolite content and the initial concentration of the liquid inoculant of the *Bacillus* consortium. The BCN-C was formulated using 0.2% liquid inoculant and 0.4 % zeolite, while BCN-G contained 0.4% liquid inoculant and 0.2% Zeolite.

Table 1. The dosage of fertilizer for each treatment

| Codes | Fertilizer Treatments | Dosage per plant |
|---|---|---|
| A | Control without fertilizers | - |
| B | NPK recommended dosage (100%) | 0.5 g |
| C | Bacillus-consortium liquid inoculant | 10 mL |
| D | BCN-C 100% | 0.50 g |
| E | BCN-C 75% | 0.375 g |
| F | BCN-C 50% | 0.25 g |
| G | BCN-G 100% | 0.50 g |
| H | BCN-G 75% | 0.375 g |
| I | BCN-G 50% | 0.25 g |

*100% is a recommended dosage equal to 1,000 kg/ha; BCN-C and BCN-G are Bacillus-coated NPK formulas C and G

The growth substrate was prepared by mixing 450 g of Andisol soil and 20 g of cow manure in a plastic container. The substrates were left overnight to reach field capacity water content before transplanting an 18-day-old tomato seedling. All potted tomatoes were grown in the greenhouse for 6 weeks in the field capacity of water content. The NPK, *Bacillus*-based liquid inoculant, and BCN were applied in split applications in a balanced amount during the vegetative stadia at five and 17 days after transplanting (Wabela *et al.* 2018)

The growth parameters, including plant height and leaf number, were analyzed at 2, 4, and 6 weeks after transplanting, while the root length, *Bacillus* population, and biomass were measured at 6 weeks. The *Bacillus* population in the rhizosphere was counted using the serial dilution plate technique with TSA (Lu *et al.*, 2018). The culture was heated at 80 °C for 20 minutes to kill vegetative cells in the endospore count (Tian *et al.*, 2022). All plate agars were incubated at 30 °C for two days.

In the sixth week, the roots were separated from the shoots; the roots were washed under tap water, dried with a towel and filter paper, and air-dried for 30 minutes before weighing, while the shoots were immediately weighed. The shoots and roots were separately wrapped in paper and stored in the oven at 60 °C for two days to weigh the dry mass. All plant and microbial data were subjected to a one-way analysis of variance (ANOVA) at $p<0.05$ and continued to the Duncan Multiple Range Test at $p<0.05$ for the significant ANOVA. All statistical analyses were performed using IBM SPPP ver. 26.

## RESULTS AND DISCUSSION

### *Bacillus* Consortia Growth and Endospore Population in Liquid Culture

The number of *Bacillus* vegetative and endospores varied depending on the composition of the media and incubation time (Figure 1). Mirel's medium supported cell proliferation more in the *Bacillus* consortium up to day nine than other media. The vegetative form constantly increased from three to nine days in *Bacillus* media. Meanwhile, in Yuniarti's and Mirel's media, the population was reduced on day six, although on day nine, both media boosted the vegetative cell growth.

A higher endospore count is detected in *Bacillus* and Yuniarti's media. The specific *Bacillus* media is formulated for *Bacillus* (ATCC Medium 552) vegetative growth, and it cannot support endospore growth. However, the medium proposed by Yuniarti *et al.* (2019) and Mirel (Mirel *et al.,* 2000) is designed to induce sporulation, support endospore population up to day nine.

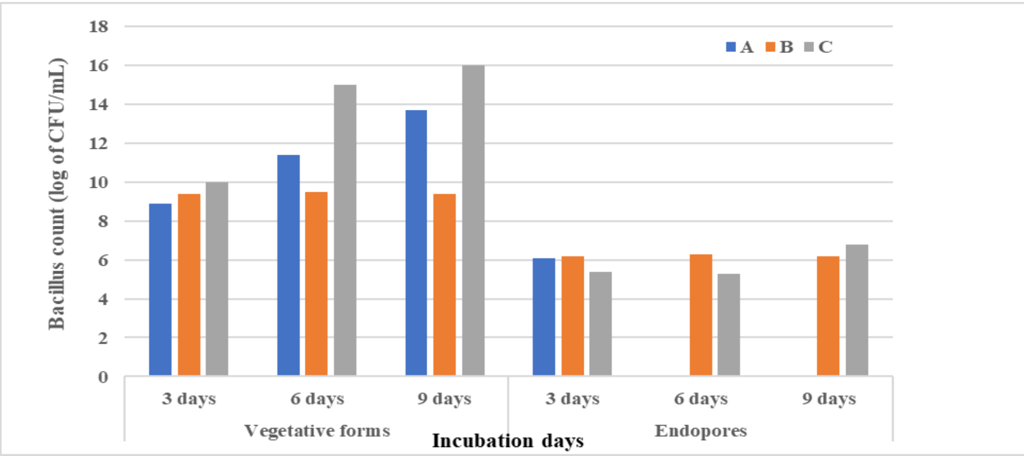


Figure 1. The population of vegetative form and endospore of *Bacillus* grown in *Bacillus*-specific (A), Yuniarti's (B), and Mirel's (C) media during 9-day incubation

The *Bacillus* endospore was only detected on day three in the *Bacillus*-specific medium, which indicated that the medium did not support sporulation. The number of endospore of Mirel's medium increased by one log from day three to day nine. The highest vegetative form was found in Mirel's medium, which had up to 6 logs of CFU/mL on day nine. In this research, the composition of the growth medium determined the vegetative form and endospore population. The composition of the *Bacillus* specific medium (Atlas, 2010) consists of various defined organic substances, while the Yuniarti and Mirel media are a nutrient-broth-based medium enriched with inorganic minerals. *Bacillus* sporulation requires mineral salt, including cationic calcium, magnesium, and manganese (Sinnelä *et al.*, 2019), which are contained in the Yuniarti and Mirel media (Yuniarti *et al.*, 2019).

The results showed that Mirel's media, which is formulated to induce sporulation, increased the endospore count. Manganese cations are crucial in initiating and continuing sporulation (Sinnelä *et al*., 2019); $Ca^{2+}$ is required to improve the endospore's heat resistance (Marquis & Bender, 1985). Meanwhile, $Mn^{2+}$, $Mg^{2+}$, $Zn^{2+}$, and $Ca^{2+}$ induced *Bacillus* sporulation and prevented spontaneous germination (Cho & Chung. 2020). Therefore, Mirel's media that contained $Mg^{2+}$, and $K^{+}$ allowed *Bacillus* to undergo better sporulation than other media. Therefore, the liquid culture of *Bacillus* for the following study was prepared using Mirel's media.

**Effect of BCN Application on Tomato Growth**

Based on the variance analysis, the application of BCN showed to be significantly affected the tomato shoot height at four and six weeks after planting but not at two weeks after planting. In the second week after planting, the average height of BCN-treated plants was 21.9 cm; statistically, it was not different from the control, conventional NPK, and *Bacillus* liquid inoculant (Table 2). The BCN treatment, except for 50% of BCN-C, can increase the plant height 4 and 6 weeks after transplanting. In week six, the application of BCN-C 100% resulted in the highest plant, although it was not too significantly different with BCN-C 75%. Based on DMRT, plants that received BCN-G 100% had a similar height to plants treated with BCN-G 75%. Tomatoes treated with half a dosage of BCN showed lower shoot height than plants with a higher BCN dosage.

Table 2. Effect of *Bacillus*-coated NPK on the shoot height of tomato plant up to six weeks after transplanting.

| Fertilizer treatments | Shoot height of tomatoes (cm) | | |
|---|---|---|---|
| | 2 WAT* | 4 WAT | 6 WAT |
| Control | 17.9 | 40.0 a | 48.7 a |
| NPK | 22.9 | 48.5 b | 63.3 bcd |
| *Bacillus* liquid inoculant | 21.0 | 42.6 ab | 56.4 abc |
| BCN-C 100% | 21.8 | 48.7 b | 71.0 d |
| BCN-C 75% | 23.1 | 50.4 b | 69.8 cd |
| BCN-C 50% | 17.9 | 33.7 a | 53.7 ab |
| BCN-G 100% | 22.9 | 49.8 b | 65.4 bcd |
| BCN-G 75% | 23.3 | 47.0 b | 65.0 bcd |
| BCN-G 50% | 22.4 | 45.3 b | 59.2 abc |

Numbers in a column followed by similar letters were not significantly different based on DMRT at p<0.05. WAT=Week after transplanting, BCN-C= *Bacillus*-coated NPK formula C, BCN-G=*Bacillus* coated NPK formula G

The control plant continuously had a lower shoot height at each measurement time than other treatments. Nonetheless, using NPK fertilizer resulted in the same plant height as applying the recommended and three-quarter dosage of BCN. At six WAP, the shoot height of the plant treated with *Bacillus* liquid inoculant did not differ from that of plants receiving BCN-G 50% (Table 2). The similar plant height indicated that the *Bacillus* in the liquid inoculant might be provided N and P as much as BCN-G, which contained higher *Bacillus* than BCN-C. The BCN-G contained zeolites that allow slow-released N and P; low N and P in soil promote the N fixation and P solubilization, respectively. The study revealed that 75% BCN can replace 100% NPK fertilizer, while 50% BCN-G can take over the *Bacillus* liquid fertilizer without plant height change.

In addition to the plant height, the leaf number was also significantly affected by BCN at all observation weeks based on analysis of variance. Different fertilizer treatments significantly changed the leaf number of tomatoes (Table 3). In the second week, reducing BCN-C to 50% can cause a lower leaf number than the control plant, but the leaf number of plants with 50% BCN-C in line with control. Starting 4 weeks after planting, introducing any formulas of BCN at both 100% and 75% of the recommended dosage resulted in the same leaf number. At week six, plants receiving all dosages of BCN-C and complete and three-quarters of dosages of BCN-G had higher leaf numbers than untreated plants and plants treated with Bacillus liquid fertilizer. Nonetheless. 50% BNC reduced the compound leaves compared to the NPK fertilizer.

A higher population of Bacillus in 75% and 100% BCN compared to that of 50% BCN provided sufficient N, P, and phytohormones for leaf development. Nitrogen and phosphorus fertilization are reported to correlate with photosynthetic activity, leaf mass, and area index (Siedliska *et al*., 2021; Qu *et al*., 2022). Cytokinins released by *Bacillus* in soil play a central role in cell division and leaf expansion (Wu *et al*., 2021). In the preliminary study before the experiment, all *Bacillus* species produced 4.47-7.35 ppm Kinetin and 0.69-7.77 mg/l Zeatin (unpublished data).

The change in the number of leaves due to BCN is consistent with the dynamics of plant height. Plants treated with specific BCN have better plant growth than the control and the same growth as the recommended dose of conventional NPK. The100% BCN-C, as well as 75% and 100% BCN-G can replace

100% NPK fertilizer to boost plant growth. Meanwhile, 100% BCN-C and 75% BCN-G could alter NPK fertilizer. The NPK replacement by 75% BCN determined that the *Bacillus* plays a role in providing the N and P, and may be K. In this study, the ability of *Bacillus* to control the K provision in soil was not studied.

Table 3. Effect of *Bacillus*-coated NPK on the compound leaf number of tomatoes up to six weeks after transplanting

| Fertilizer treatments | Number of compound leaves of tomatoes | | |
|---|---|---|---|
| | 2 WAT | 4 WAT | 6 WAT |
| Control | 6.0 ab | 8.5 a | 12.1 ab |
| NPK | 6.1 abc | 10.3 abc | 16.3 cde |
| Bacillus liquid inoculant | 6.4 abc | 9.1 ab | 10.6 a |
| BCN-C 100% | 6.1 abc | 11.5 bc | 17.2 de |
| BCN-C 75% | 6.3 abc | 10.9 abc | 19.8 e |
| BCN-C 50% | 5,13 a | 8.7 a | 14.9 bcd |
| BCN-G 100% | 7.6 cd | 11.9 c | 13.8 bcd |
| BCN-G 75% | 8.1 d | 12.0 c | 16.7 de |
| BCN-G 50% | 6.9 bcd | 9.5 abc | 12.7 abc |

Numbers in a column followed by similar letters were not significantly different based on DMRT at p<0.05. WAT=Week after transplanting, BCN-C= *Bacillus*-coated NPK formula C, BCN-G=*Bacillus* coated NPK formula G

**Plant Biomass**

Analysis of variance showed that the BCN treatment influenced the dry weight of tomato plants. Table 4 shows that plants treated with a specific dosage of BCN have higher dry weight (DW). Nonetheless, the root and shoot DW of tomatoes treated with 50% BCN were not different from the control and *Bacillus* liquid inoculant treatment. Introducing 100% BCN-C or BCN-G produced a similar root DW compared to the recommended dosage of NPK 16-16-16. Meanwhile, plants treated with lower dosages of any BCN had reduced DW than plants that received NPK.

Coating NPK fertilizer with *Bacillus* liquid inoculant in general give positive impacts to the vegetative growth of tomatoes in Andisol. Plant growth and biomass of the plant with BCN were higher than those of the control plant. This experiment showed that BCN with formula C improves plant growth better than the G formula. The recommended dose of NPK produced plants with the same growth parameters as BCN at 100% and 75%. In lettuce, NPK 16-16-16 fertilizer resulted in comparable growth traits with the recommended BCN (Hindersah *et al.*, 2023). The ability of 50% of BCN to support plant growth decreased since soil contains low N, P, and K. Without enough nutrients from BCN fertilizer, the soil cannot provide sufficient plant nutrients.

Table 4. Effect of *Bacillus*-coated NPK on the dry weight of tomato plants up to six weeks after transplanting.

| Fertilizer Treatment | Dry weight of tomato plants (g) | |
|---|---|---|
| | Roots | Shoots |
| Control | 1.9 ab | 1.8 a |
| NPK | 2.6 abc | 3.6 c |
| Bacillus liquid inoculant | 1.2 a | 2.0 a |
| BCN-C 100% | 3.9 c | 3.4 bc |
| BCN-C 75% | 2.9 abc | 3.6 c |
| BCN-C 50% | 1.9 ab | 2.0 a |
| BCN-G 100% ** | 2.9 abc | 3.3 bc |
| BCN-G 75% | 3.3 bc | 3.1 bc |
| BCN-G 50% | 1.9 ab | 2.4 ab |

Numbers in a column followed by similar letters were not significantly different based on DMRT at p<0.05. BCN-C= *Bacillus*-coated NPK formula C, BCN-G=*Bacillus* coated NPK formula G

Before treatment, the soil (Andisols) contained extremely low $P_2O_5$ (2.49 mg/kg) and an extremely high concentration of $P_2O_5$ (96.56 mg/100 g). Increased plant growth was recorded after BCN application compared to NPK because all *Bacillus* used in this study produce organic acid and phosphatase (Risanti *et al.*, 2025) that effectively provide the available P in soil with a limited content of available P (Fitriatin *et al.*, 2022). A consortium of four *Bacillus* species synthesized organic acids, including lactic, malic, citric, oxalic, tartaric, and phytase (Risanti *et al.*, 2025). P availability is the main factor for P-solubilizing microbes to provide available inorganic. The inhibition of P solubilization by phosphate-solubilizing microbes in soil with high available P has been reported (Fitriatin *et al.*, 2021).

The mechanism of plant growth enhancement by *Bacillus* is possibly through the phytohormone produced by the bacterial consortium coated onto NPK fertilizer. Phytohormones are significantly involved in regulating root and shoot growth. Preliminary studies conducted before the experiment showed that each *Bacillus* species used in this experiment synthesizes IAA, CKs (Kinetin and Zeatin), and Gas (unpublished data). It is suggested that *Bacillus* can synthesizes more cytokinins (CKs) than indole-3-acetic acid (IAA); higher CKs levels over IAA induce shoot growth and decrease apical dominance, which represses axillary bud formation (Azizi *et al.*, 2015).

### *Bacillus* Population in the Rhizosphere

Based on ANOVA, all fertilizer treatments, including any dose and type of BCN, did not change the *Bacillus* count in the rhizosphere (Table 5). Slightly insignificant reduction of vegetative forms was found in the rhizosphere of plants receiving a lower dosage of BCN-C, and 75% of BCN-G was determined. *Bacillus* population in Table 5 did not differ between indigenous and exogenous Bacillus. The result showed that the exogenous Bacillus did not outgrow the indigenous one. Table 2 shows that the exogenous *Bacillus* enabled plants to grow better than the control, which verified that exogenous Bacillus has the superior function of boosting plant growth.

*Bacillus* is among the dominant bacterial genera in soil and has enormous genetic and metabolic diversity (Liu *et al.*, 2019) The count of vegetative and endospore forms in Table 4 provides only the general *Bacillus* population which did not differentiate between *Bacillus* species and their metabolic functions.

Table 5. Effect of *Bacillus*-coated NPK on the population of vegetative cells and endospores of *Bacillus* in the rhizosphere of 6-week-old tomatoes.

| Fertilizer treatment | *Bacillus* count (log of CFU/g) | |
|---|---|---|
| | Vegetative forms | Endospores |
| Control | 10.10 a | 4.50 a |
| NPK | 10.12 a | 4.40 a |
| Bacillus LI* | 10.07 a | 4.53 a |
| BCN-C 100%** | 10.02 a | 4.42 a |
| BCN-C 75% | 9.91 a | 4.57 a |
| BCN-C 50% | 9.90 a | 4.49 a |
| BCN-G 100% ** | 10.02 a | 4.42 a |
| BCN-G 75% | 9.96 a | 4.56 a |
| BCN-G 50% | 10.07 a | 4.65 a |

Numbers in a column followed by similar letters were not significantly different based on DMRT at $p<0.05$. BCN-C= *Bacillus*-coated NPK formula C, BCN-G=*Bacillus* coated NPK formula G

The population of *Bacillus* in the rhizosphere of tomatoes has rarely been studied. The Bacillus enumeration in the tea rhizosphere showed that *B. subtilis* and *B. mycoides* populations were up to $3.9 \times 10^6$ and $10^7$ CFU/g, respectively (Pandey & Palni, 1997). Before the application of BCN, the population of vegetative forms and endospores of *Bacillus* was 7.2 and 2.6 log of CFU/g, respectively. The number is increasing after the treatment. The *Bacillus* count in the tomato's rhizosphere (Table 5) was relatively higher than before application, verifying that the rhizosphere supports *Bacillus* proliferation, possibly caused by sufficient nutrients from root exudation and fertilizer. However, the proliferation of exogenous *Bacillus* from the BCN could not be analyzed since the serial dilution plant method did not separate the exogenous from indigenous *Bacillus*.

## CONCLUSION

The population of the *Bacillus* vegetative form was determined by broth composition, while the endospores increased in broth enriched with mineral salts. The recommended and three-quarter dosage of *Bacillus*-coated NPK (BCN) resulted in the same plant height, leaf number, and dry weight of tomatoes grown with conventional NPK 16-16-16. Plants treated with both BCN-C and BCN-G formulation dosages have higher plant growth traits than plants that received no fertilizer. Nevertheless, the BCN application did not change the rhizosphere's *Bacillus* vegetative forms and endospores. The comparable plant growth of tomatoes treated with conventional NPK and 100% and 75% of BCN is promising for reducing the NPK fertilizer dosage. The results determine the prospect of using *Bacillus*-coated NPK fertilizer without reducing tomato growth. Further research concerning the *Bacillus* liquid fertilizer formulation as a base ingredient for the BCN is required to increase the endospore population. Moreover, further experiments are needed to test the BCN effect on tomato yield.

## ACKNOWLEDGMENT

Pupuk Indonesia (PI) Holding Company funded this collaborative study with the Faculty of Agriculture, Universitas Padjadjaran. The authors thank Fasa Aditya Hanindipto and Gita Bina Nugraha, from the Indonesian Fertilizer Research Institute of PI.

## REFERENCES

Airlangga, TA, N Matsue, E Hanudin, and E Johan. 2020. Phosphate adsorption capacity of allophane from two volcanic mountains in Indonesia. Journal of Tropical Soils. 25(1): 39-

46. DOI: http://dx.doi.org/10.5400/jts.2020.v25i1.39-46

Atlas, RM. 2010. Handbook of Microbiological Media, Fourth Edition. CRC Press. Boca Raton, Florida. 2,034 pp.

Cho WI, and MS Chung. 2020. *Bacillus* spores: A review of their properties and inactivation processing technologies. Food Science and Biotechnology. 29:1447–1461. https://doi.org/10.1007/s10068-020-00809-4

Azizi, P, MY Rafii, M Maziah, SNA Abdullah, MM Hanafi, MA Latif, AA Rashid, and M Sahebi. 2015. Understanding the shoot apical meristem regulation: A study of the phytohormones, auxin and cytokinin, in rice. Mechanisms of Development. 135: 1-15. https://doi.org/10.1016/j.mod.2014.11.001.

Balderas-Ruíz, KA, CI Gómez-Guerrero, MA Trujillo-Roldán, NA Valdez-Cruz, S Aranda-Ocampo, AM Juárez, E Leyva, E Galindo, and L Serrano-Carreón. 2021. *Bacillus velezensis* 83 increases productivity and quality of tomato (*Solanum lycopersicum* L.): Pre and postharvest assessment, Current Research in Microbial Sciences. 2: 100076. https://doi.org/10.1016/j.crmicr.2021.100076.

Chandini, RK, R Kumar, and P Om. 2019. The Impact of chemical fertilizers on our environment and ecosystem. Chief Education. 35:69. https://www.researchgate.net/publication/331132826

Etesami, H, BR Jeong, and BR Glick. 2023. Potential use of *Bacillus* spp. as an effective biostimulant against abiotic stresses in crops—A review. Current Research in Biotechnology. 5:100128, https://doi.org/10.1016/j.crbiot.2023.100128

Fitriatin, BN, AP Amanda, NN Kamaluddin, FH Khumairah, ET Sofyan, A Yuniarti, and T Turmuktini. 2021. Some soil biological and chemical properties as affected by biofertilizers and organic ameliorants application on paddy rice. Eurasian Journal of Soil Science. 10 (2):105 – 110. doi: https://doi.org/10.18393/ejss.829695

Fitriatin, BN, O Mulyani, D Herdiyantoro, TA Alahmadi, and M Pellegrini M. 2022. Metabolic characterization of phosphate solubilizing microorganisms and their role in improving soil phosphate solubility, yield of upland rice (*Oryza sativa* L.), and phosphorus fertilizers efficiency. Frontier in Sustainable Food System. 6:1032708. doi: 10.3389/fsufs.2022.1032708

Ghaffarpour, Z, M Fakhroleslam, and M Amidpour. 2024. Calculation of energy consumption, tomato yield, and electricity generation in a PV-integrated greenhouse with different solar panels configuration. Renewable Energy. 229: 120723. https://doi.org/10.1016/j.renene.2024.120723

Hayatsu, M, C Katsuyama, and K Tago. 2021. Overview of recent research on nitrifying microorganisms in soil. Soil Science and Plant Nutrition. 67(6): 619–632. https://doi.org/10.1080/00380768.2021.1981119

Hindersah, R, MR Setiawati, P Suryatmana, BN Fitriatin, AF Hanindipto, GB, RR Risanti and P Asmiran. 2023. Effect of NPK and *Bacillus*-coated NPK fertilizer on biomass, nutrient content in soil and nutrient uptake by lettuce. Jurnal Kultivasi. 22(1):77-84. DOI: http://dx.doi.org/10.24198/kultivasi.v22i1.43608

Hindersah, R, P Suryatmana, BN Fitriatin, P Asmiran, A Nurbaity, and MR Setiawati. 2024. Bacterial-coated urea to reduce the use of urea fertilizer in potato cultivation. Jurnal Penelitian Pertanian Terapan. 24(1):78-84. https://doi.org/10.25181/jppt.v24i1.2750

Hossain, ME, S Shahrukh, and SA Hossain. 2022. Chemical fertilizers and pesticides: Impacts on soil degradation, groundwater, and human health in Bangladesh. Pp 63–92 in Environmental Degradation: Challenges and Strategies for Mitigation. (VP Singh, S Yadav, KK Yadav, and RN Yadava, Eds). Springer, Cham. https://doi.org/10.1007/978-3-030-95542-7_4.

Jensen, CNG, JKY Pang, M Gottardi, SK Kračun, BA Svendsen, KF Nielsen, ÁT Kovács, L Moelbak, L Fimognari, S Husted, and A Schulz. 2023. *Bacillus subtilis* promotes plant phosphorus (P) acquisition through P solubilization and stimulation of root and root hair growth. Physiologia Plantarum. 176(3): e1433. https://doi.org/10.1111/ppl.14338

Liu, J, X Cui, Z Liu, Z Guo, Z Yu. Q Yao, Y Sui, Jian Jin, X Liu, and G Wang. The diversity and geographic distribution of cultivable bacillus-like bacteria across black soils of Northeast China. Frontiers in Microbiology. 10:1424. DOI: 10.3389/fmicb.2019.01424.

Marquis, RE, and GR Bender. 1985. Mineralization and heat resistance of bacterial spores. Journal of Bacteriology. 161:789–791. doi: 10.1128/jb.161.2.789-791.1985

Mirel, DB, WF Estacio, M Mathieu, E Olmsted, J Ramirez, and LM Ma´Rquez-Magan. 2000. Environmental Regulation of *Bacillus subtilis* sD-dependent gene expression. J. Bacteriol, 182(11): 3055–3062

Pandey, A, and LM Palni. 1997. Bacillus species: the dominant bacteria of the rhizosphere of established tea bushes. Microbiology Research. 152(4):359-65. doi: 10.1016/S0944-5013(97)80052-3. DOI: 10.1016/S0944-5013(97)80052-3

Poveda, K, and F González-Andrés. 2021. *Bacillus* as a source of phytohormones for use in agriculture. Applied Microbiology and Biotechnology. 105(23):8629-8645. doi: 10.1007/s00253-021-11492-8.

Qu, F, T Peng, Y Jia, M Yang, X Meng, S Mao, D Zhou, X Hu, 2021. Adjusting leaf nitrogen allocation could promote photosynthetic capacity, and nitrogen accumulation in *Cucumis sativus* L. Environmental and Experimental Botany. 198:104855. https://doi.org/10.1016/j.envexpbot.2022.104855.

Risanti, RR. R Hindersah, BN Fitriatin, P Suryatmana, IP Maksum, MR Setiawati, FA Hanindipto, and GB Nugraha. 2025. Exploring the *Bacillus* from vegetable rhizosphere for plant growth. Journal of Ecological Engineering. 26(1):109-120. DOI: https://doi.org/10.12911/22998993/195286

Saeid, A, E Prochownik, and J Dobrowolska-Iwanek. 2018. Phosphorus solubilization by *Bacillus* species. Molecules. 23(11):2897. doi: 10.3390/molecules23112897

Salsabila, N, R Hindersah, and BN Fitriatin. 2024. Enhancing Broccoli growth by *Bacillus* rhizosphere bacteria. Current Research in Agricultural Sciences. 11(2): 56-63. DOI: https://doi.org/10.18488/cras.v11i2.3945

Samaras, A, E Roumeliotis, P Ntasiou, G Karaoglanidis. 2021. *Bacillus subtilis* MBI600 promotes growth of tomato plants and induces systemic resistance contributing to the control of soilborne pathogens. Plants. 10(6):1113. doi: 10.3390/plants10061113

Siedliska, A, P Baranowski, J Pastuszka-Woźniak, M Zubik, and J Krzyszczak. 2021. Identification of plant leaf phosphorus content at different growth stages based on hyperspectral reflectance. BMC Plant Biology. 21:28. https://doi.org/10.1186/s12870-020-02807-4

Singh, RK, P Singh, HB Li, QQ Song, DJ Guo, MK Solanki, KK Verma, MK Malviya, X-P Song, P Lakshmanan, L-T Yang, and Y-R Li 2020. Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: A comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp. BMC Plant Biology. 20: 220 https://doi.org/10.1186/s12870-020-02400-9

Singh, J, SK Sandal, A Yousuf, and PS Sandhu. 2023. Effect of drip irrigation and fertigation on soil water dynamics and productivity of greenhouse tomatoes. Water. 15(11): 2086. https://doi.org/10.3390/w15112086

Sinnelä, MT, YK Park, JH Lee, KC Casey, Y-W Kim, H-J Hwang, and J-H Mah. 2019. Effects of calcium and manganese on sporulation of *Bacillus* species involved in food poisoning and spoilage. Foods. 8: 119; doi:10.3390/foods8040119

Soil Survey Staff. 2014. Keys to Soil Taxonomy, Twelfth Edition. Washington DC: Natural Resources Conservation Service-United States Department of Agriculture.

Subhan, N Nurtika, dan N Gunadi. 2009. Respons tanaman tomat terhadap penggunaan pupuk majemuk NPK 15-15-15 pada lanah Latosol pada musim kemarau. Jurnal Hortikultura. 19(1): 40-48. DOI: 10.21082/jhort.v19n1.2009.p%p

Suwarto, S, dan H Muhammad. 2023. Efektivitas *Bacillus subtilis* QST713 sebagai plant growth promoting rhizobacteria pada tanaman kubis (*Brassica oleracea*). Jurnal Hortikultura Indonesia. 14(2):118–125. https://doi.org/10.29244/jhi.14.2.118-125

Tian, Z, L Ho, L Hou, M Hu,Y Gao, D Li, B Fan, F Wang, and Shuying Li. 2022. Optimization of sporulation conditions for Bacillus subtilis BSNK-5. Processes. 10(6):1133. https://doi.org/10.3390/pr10061133.

Wabela, W. 2018. Effects of split application of nitrogen fertilizer on furrow irrigated tomato production. Journal of Natural Sciences Research. 08(11):19-22.

**Wu,** W, K Du, X Kang, and H Wie. 2021. The diverse roles of cytokinins in regulating leaf development. Horticultural Research. 8: 118. https://doi.org/10.1038/s41438-021-00558-3

Yue, Z, C Chen, Y Liu, X Chen, Y Chen, C Hu, M Zheng, J Zhang, L He, Ke Ma, and Z Sun. 2023. Phosphorus solubilizing *Bacillus altitudinis* WR10 alleviates wheat phosphorus deficiency via remodeling root system architecture, enhancing phosphorus availability, and activating the ASA-GSH cycle. Plant Soil. 492:367–379. https://doi.org/10.1007/s11104-023-06180-7

Yuliani, N, E Hanudin, and BH Purwanto. 2017. Chemical characteristics and morphology of amorphous materials derived from different parent materials from Central Java, Indonesia. International Journal of Soil Science. 12(2):54-64. DOI: 10.3923/ijss.2017.54.64.

Yuniarti, A, NB Arifin, M Fakhri, dan AM Hariati. 2019. Produksi spora dan efisiensi sporulasi *Bacillus subtilis* dengan suplementasi mangan dari sumber yang berbeda. Jurnal Ilmu Perikanan dan Kelautan. 11(2):51-58. https://doi.org/10.20473/jipk.v11i2.15250.