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Growth response of tomatoes to application of bacterial-coated NPK fertilizer in a pot experiment

Abstract. Nitrogen-fixer and phosphate-solubilizer bacteria increase the chemical-fertilizer efficiency and soil health. Bacterial-coated NPK fertilizer is a novel approach for intensifying biofertilizer application in vegetable production. The study aimed to observe the compatibility between two *Azotobacter* species and two *Bacillus* species, their population on two formulations of bacterial-coated NPK fertilizer (BCN), and the effect of BCN dose on growth, N and P content, and their uptake in tomato shoots. The compatibility test was performed using the streak method. The population of *Azotobacter* and *Bacillus* were counted for two formulations of NPK fertilizer coated by solid biofertilizer (5% and 10%) and zeolite (1% and 5%). The pot experiment was conducted in a randomized block design with four treatments and six replications. The treatments were 100% recommended doses of conventional NPK fertilizer (700 kg/ha) and 100%, 80%, and 60% doses of BCN. The results showed four bacterial species were compatible, indicated by synergistic growth on the plate agar. The BCN formula using 5% liquid inoculant and 5% zeolite has higher cell viability. The BCN enhanced stem thickness and leaves number but did not change the plant height, dry weight, N and P content, and their uptake in shoots. Applying 60% of BCN caused greater stem thickness and leaf number. Despite being insignificantly different from another treatment, that dose increased the biomass and the shoot uptake of N and P. The NPK fertilizer coated by *Azotobacter* and *Bacillus* has the potency to increase tomato growth and NPK fertilizer dose.

Keywords: *Azotobacter* · *Bacillus* · Compatibility · Nutrient uptake · Plant growth

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Introduction

Tropical soils are dominated by low available nitrogen (N), phosphor (P), and potassium (K) due to high soil decomposition and mineral weathering. Nitrate (NO_3^-) leaching from agricultural land is a major problem. Nitrogen is mobile in soil, quickly leaching out and evaporating due to high rainfall and temperature (Widowati and de Neve, 2016). Phosphorus is an immobile nutrient due to adsorption and precipitation by soil, transformation into the organic form; and excessive P fertilizer augments P loss through leaching, runoff, and erosion (Alakeh et al., 2022). To effectively address the N and P needs of plants and enhance soil productivity, vegetable growers commonly depend on chemical fertilizers, including NPK compound fertilizer. However, the effectiveness and the price of chemical fertilizers have increased. Biofertilizers are now considered part of fertilization since they can provide nutrients and phytohormones for plant growth.

West Java Province is Indonesia's most significant tomato producer; according to BPS (2023), 272,961.5 tons of tomatoes have been produced in West Java in 2022. Despite lower inorganic fertilizer effectivity use, growers rely on NPK fertilizer to provide available nutrients in the soil and boost tomato yield. Tomato plants treated with NPK fertilizer show increased yields (Rokhminarsi et al., 2020; Hamid et al., 2024; Daroini et al., 2024). Researchers agree that coating NPK fertilizer enhances its effectiveness and plant yield (Jadon et al., 2018; Wei et al., 2020) and reduces N leaching and P fixation. The tomato growers in West Java are familiar with NPK applications, but they are not familiar with the use of biofertilizers as coating agents. Therefore, coating the NPK with biofertilizer is promoted.

Generally, chemical fertilizers are coated with organic or inorganic substances such as sulfur, chitosan, and biochar (Adil et al., 2021; Danarto et al., 2017; Zulaehah and Sukarjo, 2017). Coating the NPK compound with soil-beneficial microbes is rarely reported. However, urea coated with organic matter enriched with beneficial microbes has already been developed and studied (Zulaehah, and Sukarjo, 2017; Hindersah et al., 2021a). According to SNI 2803:2012, the water content of solid NPK fertilizer is less than 3%. Coating the NPK with

manure-based solid fertilizer can increase its water content. According to Ministry of Agriculture Regulation no 261/KTPS/SR.310/M/4/2019, the water content of organic matter enriched by microbes is 10%-25%. Including the zeolite in bacterial-coated NPK fertilizer (BCN) formulation is expected to decrease its water content since the zeolite structure allows high water adsorption (Tatlier et al., 2018). A decrease in water content might not affect the viability of *Azotobacter* and *Bacillus*, which form cysts and endospores, respectively, in a water-stressed environment (Trejo et al., 2017; Khanna et al., 2020).

The drought-resistance beneficial microbe of *Azotobacter* and *Bacillus* are essential for coating the chemical fertilizer. Both bacteria are plant growth-promoting rhizobacteria (PGPR), easily found in the vegetable rhizosphere. To withstand the drought conditions, naturally, *Azotobacter* changes the vegetative cells to cysts (Trejo et al., 2017), while *Bacillus* forms the endospores (Khanna et al., 2020). *Azotobacter* is a well-known N_2 -fixer bacteria that changes the N_2 to NH_3 non-symbiotically, while *Bacillus* is reported to solubilize the inorganic P by the organic acid they produce. Both rhizobacteria synthesize the phytohormones of indole acetic acid (IAA), gibberellins (GAs), and cytokinins (CKs) (Odoh, 2017) that prominent for signaling vital molecules for cell division and elongation, as well as vascular tissue development of plants which improves plant growth (Zhao et al., 2021; Poveda et al., 2021; Liu et al. 2023).

To assure the compatibility of *Azotobacter* and *Bacillus* in the mixed inoculant, the synergisms between them are important to study. The compatibility of species or strains is vital in formulating biofertilizers that promote plant growth. Researchers have reported the synergistic effect between soil bacteria in vitro study. The *B. subtilis* and *A. chroococcum* are compatible, so both bacteria can be used in biofertilizer formulation for tomatoes (Nath et al. 2016). The *Lysinobacillus* is overgrown by *Bacillus cereus*; both isolated from organic fertilizer (Sodiq et al., 2021). Formulation of bacterial-coated NPK should be conducted to determine the viability of both microbes on the produced fertilizer.

The adoption of biofertilizer technology by farmers is low, but chemical fertilization is a common practice in growing vegetables, including in West Java. To enhance the

biofertilizer use by local farmers, farmers can be trained to use biofertilizer application as a coating agent on the NPK fertilizer. This practice will not change the farmer's fertilizer application habit. Previous research verified that urea coated by *Azotobacter-Bacillus* consortia supports strawberry seedlings and lettuce growth in the soilless substrate, reducing the dose of urea by 50% (Hindersah et al., 2021a). The *Bacillus*-coated NPK function in the growth and yield of lettuce in pot experiments has been studied (Hindersah et al., 2023a). The ability of *Azotobacter-Bacillus* coated urea to reduce the conventional urea has been demonstrated in field lettuce (Hindersah et al., 2023b). However, the interaction between *Azotobacter* and *Bacillus*, as well as the population of both bacteria in NPK fertilizer coated with *Azotobacter-Bacillus* solid inoculant, has not been studied. This research is conducted to observe the compatibility between two *Azotobacter* species and two *Bacillus* species, the population of *Azotobacter-Bacillus*-based coated NPK fertilizer (BCN), and the effect of BCN dose on growth, N and P content and their uptake in tomato's shoots.

Material and Methods

The research was carried out from December 2021 to June 2022 and comprised of three steps experiment conducted in the laboratory and greenhouse, included: 1) A compatibility test between *A. chroococcum*, *A. vinelandii*, *B. subtilis*, and *B. megaterium*; 2) The formulation of *Azotobacter-Bacillus* coated NPK, and; 3) The

application of bacterial-coated NPK fertilizer on tomatoes grown in potted soil.

Bacterial Compatibility Test. A compatibility test has been performed on a nutrient agar plate to observe the synergistic and antagonistic effect between two species of *Azotobacter* (*A. chroococcum* and *A. vinelandii*) and *Bacillus* (*B. subtilis* and *B. megaterium*). Each bacterial species was subjected to the cross-streak test between tested species. Each bacterial species was cross-streaked on the nutrient agar at the same time. The petri dishes containing cross-streaks of bacterial isolates were incubated for 24 hours at 30°C. The colony lines and inhibition zones that appeared at the paired strains' intersection were observed and photographed.

Bacterial-coated NPK Formulation. *Bacillus* and *Azotobacter* were maintained respectively on N-free TSA Ashby agar slants. Liquid inoculants of each bacterial species were produced in a molasses-based liquid medium at room temperature for 72 hours on a 120-rpm shaker. The four bacterial liquid cultures were mixed in a balanced composition and then incubated for 72 h before solid inoculant formulations. The main ingredient of BCN was 200-mesh cow manure at 20% of water content. The formulation treatments were two compositions of zeolite, and the initial liquid inoculant included A: 1% Zeolite + 10% Liquid Inoculant and B: 5% Zeolite + 1% Liquid Inoculant (Hindersah et al., 2021b). Compost was mixed with zeolite, sterilized in a 14 cm x 23 cm aluminum bag using the autoclave, and stored for 72 hours at room temperature without sunlight exposure. The bacterial consortium liquid inoculant was injected into the carrier, mixed by hand evenly, and incubated for 72 hours.



Figure 1. Steps of bacterial coating on NPK fertilizer using a manual rotator.

The NPK 16:16:16 fertilizer was coated separately with each manure-based biofertilizer formula using a manual rotator at room temperature for 15 minutes (Figure 1). The weight ratio of NPK and biofertilizer is 10:1. Population densities of total *Bacillus* and *Azotobacter* were calculated on days 3 and 7 using the serial dilution plate method on TSA for *Bacillus* and Ashby's Mannitol agar for *Azotobacter*. The experiment was conducted in triplicate. The greenhouse experiment will utilize the BCN with a higher population of total *Azotobacter* and *Bacillus*.

Greenhouse Experiment. The greenhouse experiment was conducted at the Faculty of Agriculture, Jatinangor, West Java, from December 2021 to June 2022. The experimental site was located in a tropical area at an altitude of 752 m above sea level; the monthly temperature, relative humidity, and rainfall during the experiment were 19.3 - 28.8 °C; 56.3 - 97.0%, and 75 - 273 mm, respectively. The geographical position of the greenhouse is - 6.9164472 S and 107.770653,19 E. The pot experiment was conducted in Inceptisols soil with a pH of 4.92 (acidic). The soil contained 1.5% Organic-C (low), 0.28% total-N (medium), 5.36 in C/N (low), 6.09 mg/kg available P₂O₅ (low), 20.43 mg/100g potential-P₂O₅ (low), and 7.53 mg/100 g potential-K₂O (low); the cation exchange capacity of the soil was low (5.8 cmol/kg. In general, the soil had low fertility.

The 17-day-old seedlings of tomato cv Servo (F1) were grown in Inceptisol mixed with cow manure (23.4% organic C, 1.97% total N, 1.09 % total P₂O₅, 0.73 % total K₂O, water content of 15.57%, and C/N of 11.69). The carrier of biofertilizer used in coating the fertilizer was 200-mesh cow manure mixed with 5% of 100-mesh zeolite and 5 % of the initial concentration of *Azotobacter*-*Bacillus* liquid inoculant. This formula has a higher *Bacillus* population based on the second research step.

Experimental Design. The pot experiment was set up in a randomized block design with five treatments and six replications. The treatments were four doses of bacterial-coated NPK fertilizer (e.g., 60%, 80%, and 100% equal to 10.18 g, 14.4 g, and 18 g/plant, respectively). The 100% dose of BCN and NPK fertilizer referred to the recommended dose of NPK fertilizer (700 kg/ha), according to the West Java Agricultural Technology Research Center (Sutrisna and Surdianto, 2014).

Experimental setup. Single 17-day-old tomato seedlings were transplanted into 7 kg of soil mixed with 20% cow manure in a perforated polybag. When the plants were two weeks old, 1.5-m bamboo stakes were installed in each polybag to prevent them from falling over. All kinds of fertilizers were applied in split applications, i.e., a week before planting and at the first and fourth weeks after planting (WAP). Half a dose of fertilizer was applied before planting; another half dose was split at the first and fourth weeks. Fertilizers were put in a three-cm-deep circular hole 10 cm away from the stem and then covered with soil.

Parameters and Statistical Analysis. The plant height, stem-base diameter, and leaf number were measured at the 3rd and 4th WAP. The dry weight of the shoot and roots, shoot-to-root ratio, and N and P content of the shoot were determined in the 4th WAP when the plants initiated the generative stadia. The N and P uptake by a plant was then calculated based on the N or P content of the shoot. A normality test was performed on all datasets to ensure a normal distributed population before analyzing variance at $p < 0.05$. If the effect of the mean square of treatment on the parameter was significant, then Duncan's Multiple Range Test (DMRT) at $p < 0.05$ was performed. Statistical analysis was conducted using the Statistical Package for the Social Sciences application.

Results And Discussion

Compatibility among bacterial species. The four species of *Azotobacter* and *Bacillus* show synergism without inhibiting each other's growth (Figure 2). The compatibility or synergism of two or more inoculated bacteria is a key factor in biofertilizer formulation to ensure their synergistic effect (Asri et al., 2016). This result agrees with the synergistic growth between two *Bacillus* strains and a single strain of *Azotobacter* (Proboningrum et al., 2019).

Bacterial species interact synergistically with other species that share the same nutritional sources. Their metabolic similarity is a key driver of niche deformation between bacterial species; resource availability emerges during interaction and affects positive interactions within the species (Qiao et al., 2024). Therefore, they have cooperative behavior in a consortium and produce metabolites that fit the needs of all bacteria and mutually support their growth (Asri et al., 2016).

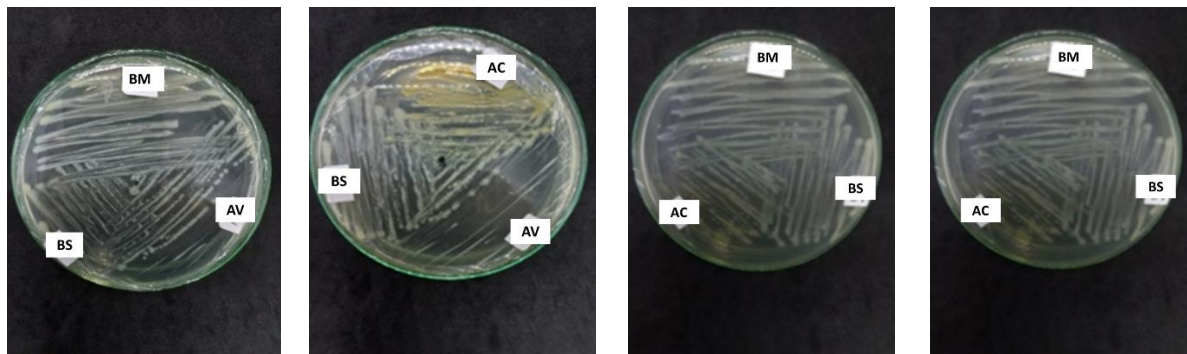


Figure 2. Synergistic growth of *A. chroococcum* (AC), *A. vinelandii* (AV), *B. subtilis* (BS), and *B. megaterium* (BM) indicated by the absence of halo zone

A pot culture experiment determined the synergism of *Azotobacter* and *Bacillus* in enhancing the germination percentage and vigor index of Chili var K-1 as well as plant growth and yield compared to single inoculation (Kanchana et al., 2014).

Population of *Azotobacter* and *Bacillus* on the coated fertilizer. The NPK fertilizer was coated with manure-based inoculant mixed with two concentrations of initial liquid inoculant. The density of *Azotobacter* and *Bacillus* in each BCN formulation was similar, but the population of *Bacillus* at seven days after coating was significantly higher (Figure 3). Therefore, the B formulation was further used for the greenhouse experiment; this formulation was 5 % liquid inoculant in a manure-based carrier mixed with 5% zeolite during fertilizer coating.

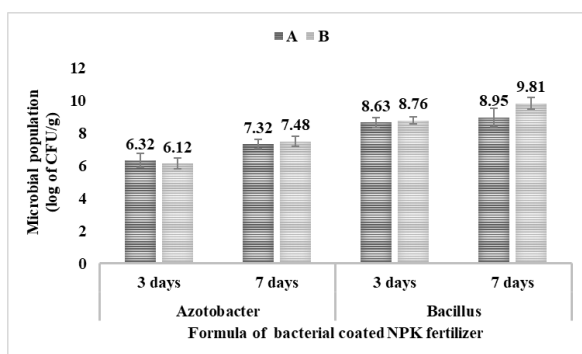


Figure 3. The population of *Azotobacter* and *Bacillus* in two formulas of bacterial-coated NPK fertilizer at 3 and 7 days after coating. A: 1% zeolite, 10% liquid inoculant; B: 5% Zeolite, 5% liquid inoculant

The role of zeolite in chemical coating processes is to maintain the water content of NPK fertilizer since the water content of manure was 20%. Drying processes in fertilizer manufacture will reduce the water content of

those fertilizers by less than 2% (Marintika et al., 2023). The NPK fertilizer requires 3% water content and the manure humidity was 20%. A mixed solid inoculant with 5% zeolite was supposed to maintain fertilizer humidity by capturing the water (Aleksandrak et al., 2020) and ensuring the viability of both bacteria. The *Azotobacter* and *Bacillus* can withstand dry conditions by forming cysts and endospores, respectively. This result reconfirmed the effectivity of 5% zeolite in maintaining cell viability in a previous study using urea (Hindersah et al., 2021a).

Plant Growth. Bacteria-coated NPK fertilizer did not change the plant height (Table 1), but 60% of BCN enhanced the stem diameter and leaves number at the 3rd and 4th WAP (Tables 2 and 3). The *Azotobacter* provides the NH_3 , which is further changed by nitrification bacteria to NO_3^- which is available for root uptake. The *Bacillus* provide the available P through unavailable-P solubilization. As biostimulants, both rhizobacteria were reported to synthesize various phytohormones. Plant growth depends on sufficient N and P, but stem development and leaf formation are also regulated by phytohormone. The GA, CK, and auxin signaling pathways in tomatoes, including their synergistic or antagonistic relationship, are essential for stem thickness (Liu et al., 2023; Peng et al., 2024). Combine factors of various phytohormones, able including abscisic acid (ABA), auxin, GAs, CKs, and Brassinosteroids (BRs), are involved in the establishment of leaf polarity, morphology, and development (Zhao et al., 2021).

In this experiment, the dry weight of the shoot (S) and root (R), as well as their S-to-R ratio (S/R), were not determined by the type and dose of NPK fertilizer (Table 4). Nonetheless, regardless of statistical analysis,

the S/R of a lower dose of BCN decreased the S/R. The lower S/R indicated that the BCN promotes more roots than shoot growth during the vegetative stage. It is likely that all bacteria in coated urea function to provide phytohormones. *Azotobacter* and *Bacillus* can synthesize growth-promoting phytohormones (Odoh, 2017). In liquid culture, *A. salinestris* AT19 and *A. chroococcum* AT25 strains produce IAA, GA3, and Zeatin in the late logarithmic phase (Rubio et al., 2013). The CK and IAA were detected in the liquid culture of *Bacillus* consortia (Hindersah et al., 2019). The effect of IAA and CK on root formation has been reported (Aloni et al., 2006); CK in the root cap promotes cytokinesis root apical dominance, while IAA promotes root development and induces vascular differentiation.

Table 1. Effect of bacterial-coated NPK fertilizer on plant height of tomatoes.

Fertilizer	Plant height (cm)		
	2 WAP	3 WAP	4 WAP
100% NPK	19.2 ± 2.91	41.4 ± 5.14	74.5 ± 3.83
100% BCN	19.1 ± 3.69	39.7 ± 7.13	72.3 ± 11.89
80% BCN	17.1 ± 2.91	39.8 ± 3.75	70.7 ± 8.87
60% BCN	20.7 ± 0.48	41.7 ± 2.31	71.8 ± 6.45

Table 2. Effect of bacterial-coated NPK fertilizer on stem diameter of tomatoes.

Fertilizer	Stem diameter (mm)		
	2 WAP	3 WAP	4 WAP
100% NPK	4.6 ± 0.5	6.7 ± 0.9a	8.2 ± 0.9a
100% BCN	4.6 ± 0.4	6.6 ± 0.9a	8.1 ± 0.8a
80% BCN	4.9 ± 0.5	6.9 ± 0.5a	8.5 ± 0.7a
60% BCN	5.3 ± 1.1	7.2 ± 0.9b	9.5 ± 1.0b

Values in the column followed by the same letter are not significantly different based on the DMR test at $p < 0.05$

Table 3. Effect of bacterial-coated NPK fertilizer on leaves number of tomatoes.

Fertilizer	Number of Leaves		
	2 WAT	3 WAT	4 WAT
100% NPK	15.4 ± 1.2a	46.2 ± 8.0b	83.3 ± 8.6a
100% BCN	15.4 ± 2.3a	42.5 ± 7.0a	80.8 ± 15.8a
80% BCN	16.5 ± 1.5ab	49.7 ± 9.3bc	84.5 ± 7.9a
60% BCN	15.4 ± 1.9a	50.3 ± 7.5c	90.5 ± 14.0b

Values in the column followed by the same letter are not significantly different based on the DMR test at $p < 0.05$

Table 4. Effect of bacterial-coated NPK fertilizer on the dry weight of tomatoes.

Fertilizer	Shoot (g)	Root (g)	Ratio S/R
100% NPK	11.05 ± 3.2	1.39 ± 0.3	8.1 ± 2.2
100% BCN	11.83 ± 2.3	1.43 ± 0.4	8.6 ± 2.0
80% BCN	10.04 ± 4.2	1.28 ± 0.5	7.3 ± 2.1
60% BCN	12.70 ± 2.4	1.75 ± 0.2	7.3 ± 1.2

BCN also did not influence the N and P content in the shoots or their uptake (Table 5). The N and P uptake of tomatoes grown with 60% BCN was higher than that of other treatments due to higher biomass, but the difference was not statistically significant. This research found that BCN reduces conventional NPK; plants receiving 10.8 g and 14.4 g BCN (equal to 720 kg/ha and 960 kg/ha) demonstrated the same dry weight and N and P status of potato shoots.

Eventually, the role of *Azotobacter* in fixing the N and *Bacillus* in solubilizing P was not clearly shown in this pot experiment since the BCN did not affect the N and P status in plants (Table 5). They might function in providing the available N and P in soil, but the different concentrations were too low to be statistically significant. Moreover, potted soil contained sufficient N and P for uptake by roots, which reduces the ability of nitrogenase to catalyze N-fixation and suppresses the function of phosphate solubilizing microbes to change the form of unavailable inorganic P to available one.

Conclusions

The four bacterial species included, *A. chroococcum*, *A. vinelandii*, *B. subtilis*, and *B. megaterium*, did not demonstrate an antagonistic effect on each other growth; this indicated that the bacteria were compatible to be formulated in bacterial-coated NPK (BCN) fertilizer. The BCN formulated by employing manure and 5% zeolite, inoculated with 5% liquid inoculant, has greater cell viability. A lower dose of said BCN formula increased the stem thickness and leaf number of tomatoes in the vegetative stage. The function of various doses of BCN to provide the N and P remained unclear since *Azotobacter*, and *Bacillus* did not affect the N and P status in the shoots of tomatoes compared to the conventional NPK fertilizer.

Table 5. Effect of bacterial-coated NPK fertilizer on N and P uptake by shoots of tomatoes

Fertilizer	N (%)	P (%)	N uptake (mg/plant)	P uptake (mg/plant)
100% NPK	6.33 ± 0.29	0.76 ± 0.15	704.9 ± 177.9	81.1 ± 17.2
100% BCN	5.90 ± 0.52	0.67 ± 0.08	687.5 ± 104.6	81.2 ± 13.6
80% BCN	6.37 ± 0.64	0.78 ± 0.15	645.8 ± 229.9	77.2 ± 24.1
60% BCN	5.97 ± 0.31	0.72 ± 0.09	760.6 ± 144.2	92.1 ± 15.4

However, plants treated with lower doses of BCN have similar biomass and N and P uptake, which showed their possibility of reducing the NPK fertilizer. The formulation of BCN might be performed by elaborating the composition of liquid inoculant and zeolite in the carrier-based inoculant, but the analysis of BCN water content is suggested to ensure the quality of BCN. Moreover, research concerning the function of BCN on plant yield is needed to prove the function of BCN in increasing the yield.

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