Bacillus-Coated Inorganic Fertilizer to Improve Plant Growth and Bacillus Count in the Rhizosphere of Broccoli

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ABSTRAK

Inovasi pupuk Bacillus pembentuk endospora berlapis NPK diharapkan dapat mengatasi rendahnya ketersediaan fosfat pada tanah Andisol. Telah dilakukan percobaan pot untuk menganalisis dampak berbagai dosis dua jenis BCN terhadap pertumbuhan dan biomassa brokoli, serta terhadap populasi Bacillus di rizosfernya. Percobaan disusun dalam rancangan blok acak untuk menguji kombinasi jenis dan dosis BCN dibandingkan dengan NPK dan inokulan cair Bacillus. Tidak ada kombinasi jenis dan dosis BCN yang mempengaruhi pertumbuhan brokoli umur 6 minggu, meskipun 100% BCN-B meningkatkan tinggi tanaman pada 2 minggu. Biomassa akar meningkat setelah aplikasi 75% BCN-A, sedangkan 100% BCN-B meningkatkan biomassa pucuk. Populasi spora meningkat pada rizosfir tanaman dengan 100% BCN-B dan 75% dari kedua jenis BCN namun total populasi sel vegetatif tidak dipengaruhi oleh BCN. Percobaan ini menyarankan penggunaan BCN untuk meningkatkan pertumbuhan brokoli.

Kata kunci: Andisols, Biomassa, Pupuk NPK, Sel Vegetatif, Spora

1. INTRODUCTION

Broccoli is not native to Indonesia but is widely cultivated in mountainous areas where the temperature meets its growth requirements. Nowadays, broccoli has high economic value and is widely consumed due to its nutritional benefits. Monoculture broccoli farming is both profitable and feasible, with a Revenue-Cost Ratio of 1.75 (Setyawan et al., 2021). Broccoli is a rich source of antioxidants; vitamins B3, B6, K, C, and E; and essential minerals such as iron, calcium, zinc, and potassium (Booth, 2021; Kim et al., 2022).

Volcanic soils, particularly Andisols, dominate the mountainous regions of Indonesia. These soils exhibit favorable physical and chemical properties for vegetable production. However, the growth of annual vegetables in Andisols is constrained by low phosphorus (P) availability due to fixation by allophane as well as aluminum and iron oxides (Airlangga et al., 2020; Poblete-Grant et al., 2020). Farmers commonly apply NPK fertilizer to improve P availability and, consequently, P uptake and broccoli yield (Puspitaeni et al., 2023). Combining inorganic NPK fertilizer with goat manure can increase the fresh weight of broccoli heads by 203% (Panjaitan & Sudiarso, 2021).

The long-term and excessive use of inorganic fertilizers, however, has been reported to degrade soil quality. Combining NPK and nitrogen fertilizers with organic matter can increase soil organic carbon, total nitrogen, and enzymatic activity (Ge et al., 2018; Zhang et al., 2022). Conversely, inorganic fertilizer application alone tends to reduce the soil C/N ratio and pH (Ge et al., 2018). Enhancing broccoli growth and yield, therefore, requires sustainable phosphorus (P) management to ensure sufficient availability for vegetative growth and flower production. Plant Growth-Promoting

Rhizobacteria (PGPR), particularly Bacillus, offer a promising alternative for maintaining or even improving soil properties while reducing dependence on inorganic fertilizers.

Key plant growth-promoting traits of Bacillus include phosphate solubilization, organic P mineralization, and phytohormone production (Fitriatin et al., 2022; Poveda & González-Andrés, 2021). Bacillus is an endosporeforming rhizobacterium capable of surviving drought and adapting to diverse environments (Etesami et al., 2023), making it advantageous for vegetable production in dryland areas with limited irrigation, such as those in Indonesia. Currently, the use of Bacillus and other beneficial soil bacteria as biofertilizers is far less intensive than the use of inorganic fertilizers.

Innovative fertilizer formulations are crucial for encouraging farmers to adopt this technology. Bacillus-coated NPK (BCN) is more practical to apply, allowing farmers to introduce inorganic fertilizer and biofertilizer simultaneously. Furthermore, BCN has the potential to reduce the required dose of conventional NPK fertilizer while maintaining Bacillus populations in the rhizosphere. We have developed two BCN formulations, but few studies have examined their performance in broccoli cultivation. Previous experiments demonstrated that a low dose of BCN had comparable effects to NPK fertilizer on lettuce growth (Hindersah et al., 2023). Pot experi-ments also suggest that BCN can potentially replace NPK 16-16-16 fertilizer for tomato production in Andisols (Hindersah et al., 2025). Therefore, this pot experiment was conducted to evaluate the effects of different doses of two types of BCN on broccoli growth and biomass, as well as on Bacillus populations in the rhizosphere.

2. MATERIALS AND METHODS

The research took place between May and October 2023 and involved a consortium of *Bacillus safensis* B1, *B. subtilis*D2, *B. altitudinis* B14, and *Bacillus* sp. E2. These bacteria were phosphate-solubilizing agents that facilitated the production of phytohormones, including indole-3-acetic acid, zeatin, and kinetin (unpublished data). The *Bacillus* liquid inoculant was formulated in a molasses-based medium enriched with peptone, beef extract, and KH_2PO_4. Two formulations of Bacillus-coated NPK (BCN) were developed using distinct compositions of liquid inoculants and zeolite, namely BCN-A with 0.2% liquid inoculant and 0.4% zeolite, and BCN-B with 0.4% liquid inoculant and 0.2% zeolite. The pot experiment was conducted in the greenhouse at the Faculty of Agriculture, located 797 m above sea level.

The soil was volcanic Andisols collected from Lembang, Bandung Barat Regency. The soil's pH was 5.8, and its texture was sandy loam with 64% sand, 33% silt, and 3% clay. It contained 3.69% organic carbon, 0.26% total nitrogen, a C/N ratio of 14, 2.49 mg/kg available phosphorus, 96.56 mg/100 g potential P_2O_5, and 166.13 mg/100 g potential K_2O. The soil's cation exchange capacity and base saturation were 34.99% and 49%, respectively

2.1 Experimental Design

The experiment tested nine treatments, including control treatments and the combination treatments of the type of NBB and their doses as follows:

A. Control without NPK and biofertilizer
B. 100 % dose of NPK
C. Liquid inoculant of Bacillus consortia, recommended dose 5 L/ha
D. 100 % dose of BCN-A
E. 75 % dose of BCN-A
F. 50 % dose of BCN-A
G. 100 % dose of BNC-B
H. 75 % dose of BCN-B
I. 50 % dose of BCN-B

Each treatment was replicated three times. The 100% dose of NPK and BCN in this experiment was 100 kg/ha. The full dose of NPK and BCN fertilizer was 2.5 g per hectare of field, based on the plant population in one hectare. The uncoated and coated NPK were applied twice during transplanting and 28 days after. The Bacillus liquid fertilizer was inoculated one week after transplanting.

2.2 Experimental Setup

The debris-free soil was air-dried and mixed with composted cow manure at a rate of 20 t/ha. A total of 500 g of the soil-manure mixture was placed in a transparent container, irrigated with 200 mL of groundwater, and left overnight. An 18-day-old seedling of Broccoli cv. was transplanted into a 5-cm-deep planting hole containing 20 g of manure and was immediately watered with 50 mL of groundwater. The potted broccoli was maintained in the greenhouse for six weeks.

The NPK and BCN were applied twice, at one and four weeks after planting. The liquid fertilizer was diluted in water (1:10) and used through soil dressing at a rate of 10 mL per plant at the time of planting and again two weeks later. Pesticides were not applied because there were no disease attacks.

2.3 Parameters and Statistical Analysis

The shoot height and leaf number were measured weekly for six weeks. At six weeks after planting, the fresh and dry weights of shoots and roots were analyzed, and the root length was measured. At the end of the experiment, the vegetative cells and spores of Bacillus were counted using the serial dilution plate method with Tryptic Soy Agar (Liu *et al*., 2018). The bacterial culture was heated for 20 minutes at 80 °C before spore counting (Tian *et al*., 2022). The plate agars were incubated for two days at 30 °C. All data were subjected to analysis of variance with $p < 0.05$, and Duncan's multiple range test was applied with $p < 0.05$ when the treatment significantly affected the specific parameter.

3. RESULTS AND DISCUSSION

The analysis of variance verified that various fertilizer treatments influenced the shoot height and leaf number of broccoli. In the first and second weeks, plants treated with 100% BCN-B exhibited significantly higher shoot height than those receiving 100% NPK, while those of 75% and 50% BCN-A were slightly lower than 100% BCN-B (Table 1).

Table 1 Shoot height of broccoli grown in Andisols treated with various doses and types of Bacillus-coated NPK fertilizer

Code	Treatments	Shoot height (cm) per week					
		1	2	3	4	5	6
A	Control	6.30 ab	8.10 ab	10.40 a	12.00 a	14.10	15.00
B	100% of NPK	6.10 ab	8.30 ab	9.90 a	11.60 a	13.00	13.10
C	Bacillus liquid fertilizer	7.70 abc	10.30 ab	11.53 a	13.00 a	13.70	14.60
D	100% of BCN-A[1]	6.00 ab	8.00 ab	10.40 a	11.13 a	12.10	12.80
E	75% of BCN-A	7.90 bc	10.00 ab	11.90 a	12.60 a	13.70	14.60
F	50% of BCN-A	7.40 abc	9.60 ab	11.10 a	12.90 a	13.50	14.60
G	100% of BCN-B[2]	8.50 c	11.90 b	11.90 a	14.10 a	14.50	16.70
H	75% of BCN-B	5.70 a	7.60 a	10.90 a	12.70 a	12.80	13.90
I	50% of BCN-B	7.20 abc	9.00 ab	10.90 a	11.44 a	12.80	14.00

Note: The values followed by the same letters were not significantly different based on Duncan's Multiple Range Test with $p<0.05$. [1]Bacillus-coated NPK formula A, [2]Bacillus-coated NPK formula B.

At three weeks after transplanting, the highest number of leaves was observed in plants with 75% BCN-A and 100% and 75% BCN-B (Table 2). Nonetheless, at four to six weeks, neither plant height nor leaf number was affected by the fertilizer treatment. At six weeks, the plant receiving 100% BCN-B had a relatively higher shoot height and number of leaves, although these differences did not reach statistical significance compared to the other treatments.

At six weeks after planting, the root lengths of broccoli plants treated with BCN did not differ from those of the control (Figure 1). Regardless of statistical analysis, the root length of plants with uncoated NPK fertilizer was lower than that of the control (without any fertilizer) and Bacillus liquid fertilizer (LF).

Table 2 Leaf number of broccoli grown in Andisols treated with various types and doses of Bacillus-coated NPK fertilizer

Code	Treatments	Leaf number per week					
		1	2	3	4	5	6
A	Control	5.94 ab	6.65 a	7.20 b	7.00 a	7.60	8.40
B	100% of NPK	6.20 b	6.80 a	6.44 ab	6.20 a	5.00	5.80
C	Bacillus liquid fertilizer	6.00 ab	7.06 a	7.06 ab	7.06 a	7.20	8.00
D	100% of BCN-A[1]	5.00 a	6.00 a	5.60 a	6.00 a	5.80	5.40
E	75% of BCN-A	6.00 ab	6.20 a	7.20 b	7.00 a	7.20	8.60
F	50% of BCN-A	5.80 ab	6.80 a	6.83 ab	7.00 a	6.80	7.60
G	100% of BCN-B[2]	6.00 ab	6.80 a	7.94 b	8.00 a	7.20	9.00
H	75% of BCN-B	5.80 ab	6.80 a	7.40 b	6.60 a	6.80	7.20
I	50% of BCN-B	6.43 b	6.65 a	7.06 ab	6.80 a	7.00	8.20

Note: The values followed by the same letters were not significantly different based on Duncan's Multiple Range Test with $p<0.05$. [1]Bacillus-coated NPK formula A, [2]Bacillus-coated NPK formula B

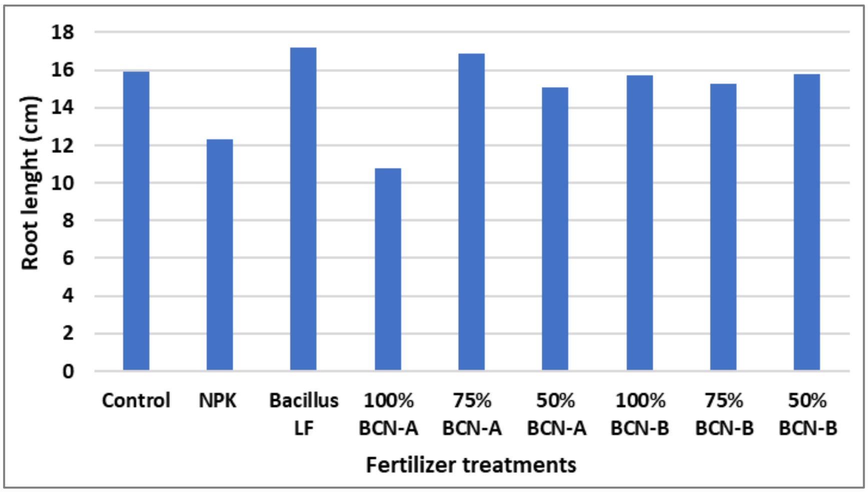

Figure 1 The root length of 6-week broccoli grown in potted Andisols with various doses and types of Bacillus-coated NPK fertilizer.

The fertilizer treatments significantly influenced the biomass of both the root and shoot, but did not affect the root length. At week six, the plants receiving 100% of NPK (16-16-16) and 75% of BCN-A exhibited the highest root fresh weight, while those receiving 75% of BCN-A and 50% of BCN-C showed the highest root dry weight (Table 3). Surprisingly, the shoot biomass of plants with 100% NPK was lower than that of the control and other treatments. Despite having similar shoot heights and numbers of leaves, the shoot fresh and dry biomass of the control plants matched that of plants treated with specific types and doses of BCN.

Figure 2 shows the same population of vegetative Bacillus cells in the rhizosphere of broccoli at 6 weeks. Nonetheless, their spore population can be increased by applying 75% of BCN-A, as well as 100% and 75% of BCN-B. In this experiment, the vegetative cell and spore counts of Bacillus in the rhizosphere were approximately 9 and 3 in a log scale, equivalent to 10^9 and 10^3 CFU/g, respectively.

Table 3 Biomass of 6-week-old broccoli plants grown in Andisols treated with various types and doses of Bacillus-coated NPK fertilizer

Code	Treatments	Fresh weight (g)		Dry weight (g)	
		Root	Shoot	Root	Shoot
A	Control	5.40 ab	12.60 abc	2.70 ab	2.50 c
B	100% of NPK	8.40 b	8.80 a	1.60 a	1.20 a
C	Bacillus liquid fertilizer	6.40 a	13.30 bc	2.30 ab	2.00 abc
D	100% of BCN-A[1]	4.00 a	9.70 ab	1.70 a	1.40 ab
E	75% of BCN-A	8.30 b	13.20 abc	3.70 b	1.90 abc
F	50% of BCN-A	7.10 ab	12.80 abc	2.70 ab	1.70 abc
G	100% of BCN-B[2]	4.70 ab	14.80 c	2.20 ab	2.10 abc
H	75% of BCN-B	6.00 a	11.70 abc	2.50 ab	1.80 abc
I	50% of BCN-B	7.70 ab	12.80 abc	3.70 b	2.40 bc

Note: The values followed by the same letters were not significantly different based on Duncan's Multiple Range Test with $p<0.05$. [1]Bacillus-coated NPK formula A, [2]Bacillus-coated NPK formula B

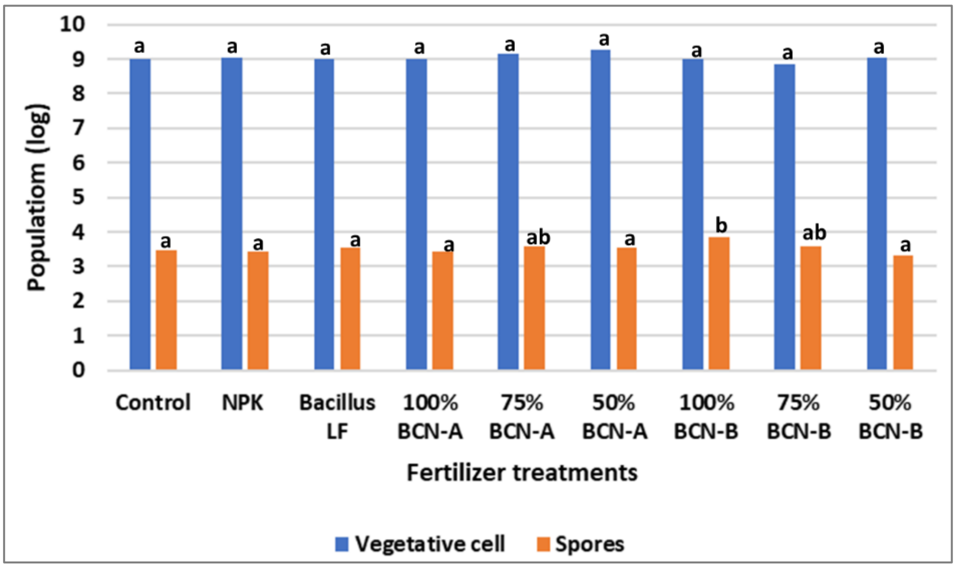

Figure 2 The vegetative cell and spore population of Bacillus in the rhizosphere of broccoli at six weeks after transplanting

The specific dose and type of BCN affected only shoot height and the number of leaves during the early growth stage. The ability of BCN to enhance plant growth is believed to relate to the viability of exogenous Bacillus in the first two weeks, and hence its activity in providing available P and phytohormones. Between 3 and 6 weeks, the Bacillus adhered to the surface of NPK might proliferate in the soil but cease its activity to promote plant growth.

This dose of BCN, along with the NPK added to each potted broccoli, equates to only 100 kg/ha, which is lower than the dose recommended by Puspitaeni *et al*. (2023). The ability of exogenous Bacillus to enhance growth lasts only up to 2 weeks, possibly related to less favorable soil conditions and the initially low population. The pH was 5.8, which is lower than the optimal range for Bacillus proliferation, 6-8; however, certain Bacillus species require a higher pH for optimal growth. The *B. subtilis* T9-05 achieves its highest growth at pH 7-8 (Sanjaya *et al*., 2023). All Bacillus species utilized in this experiment have not yet been assessed for their growth in different acidic conditions.

Although there was no effect of BCN on plant growth at six weeks, the specific BCN was able to enhance shoot and root biomass (Table 3). Photosynthesis, the fundamental process for plant growth and biomass accumulation, directly influences yield potential (Kromdijk *et al*., 2016; Khan *et al*., 2017). Nitrogen is a key component of chlorophyll, which is crucial for photosynthesis (Evans and Clarke, 2019). The BCN contained zeolites that allow N to be slowly released into the soil; moreover, Bacillus are spore-forming bacteria that may become vegetative cells during late vegetative growth. The increase in biomass at six weeks is attributed to the continuous N supply from BCN. Furthermore, it may be caused by the increasing available N provided by Bacillus through nitrogen fixation. The Nif gene, responsible for nitrogen fixation, was identified in particular Bacillus species (Jain *et al*., 2021).

Despite the minimal impact of BCN on the total Bacillus vegetative cell population, the spore population slightly increased due to specific BCN doses and types (Figure 2). Nonetheless, the rise in fungal count was gradual; the fungal count in all treatments was three log units, indicating that exogenous Bacillus did not outcompete the indigenous one in the sporulation process. The formation of spores will enhance the next phase of broccoli growth, which will be confirmed if the research continues until harvest time.

4. CONCLUSION

Compared to the uncoated NPK fertilizer, the Bacillus-coated NPK (BCN) did not affected the plant growth in terms of plant height, root length, and number of leaves. Specific BCN significantly increased plant biomass.

The application of 75% of BCN-A significantly enhanced the fresh and dry weight of roots, while higher weights of shoots and roots were noted in plants receiving 100% of BCN-B. The population of Bacillus vegetative cells in the rhizosphere was unaffected by any fertilizer treatment, including BCN. A higher spore count was observed in plants treated with 100% BCN-B and 75% of both BCN types. The pot experiment indicated that BCN shows promise for use in broccoli cultivation. However, further experimentation is needed to verify the impact of soil pH on Bacillus proliferation and its effect on broccoli yield.

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