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# Effect of NPK and Bacillus-coated NPK fertilizer on biomass, nutrient content in soil and nutrient uptake by lettuce

**Abstract.** The Inoculation of beneficial soil microbes is an effective method for lowering doses of inorganic fertilizers. This study was aimed to observe and compare the effect of doses and formulas of Bacillus-coated NPK (BCN) and conventional NPK fertilizers on biomass, major macro-nutrient in soil and their uptake by shoots of lettuce (*Lactuca sativa* L.); as well as evaluate the potency of BCN for decreasing doses of NPK fertilizers. The greenhouse experiment was set up in a randomized block design with seven treatments and five replications. The treatments included one and a half doses of recommended NPK fertilizer and two BCN fertilizer formulas; control treatment was without any fertilizer. This experiment showed that NPK fertilizer had comparable effect with BCN on growth traits; but application of NPK and coated NPK had a potency to increase the fresh weight of lettuce up to 24-45% which was in line with the increase of shoot-to-roots ratio. The potassium (K) content in soil and their uptake in lettuce shoots depend on doses and type of NPK but Nitrogen (N) and Phosphorus (P) content in soil and in shoot were not determined by treatments. The results showed that the recommended NPK dose (200 kg/ha) for lettuce can be reduced up to 50%; moreover, 50% of BCN enabled to maintain the N, P and K uptake as well as the lettuce yield.

**Keywords**: Bacillus · Biomass · Plant growth · Nutrient uptake

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## Introduction

Currently, food crops cultivation should be supported by proper fertilization methods to maintain soil health and plant productivity. In general, farmers apply inorganic fertilizer such as NPK compound and Urea for yield increment. Nonetheless, the excessive and long-term use of inorganic fertilizers, caused N evaporation and leaching; and P adsorption by clay. Acid fertilizer can reduce the soil pH and limit the availability of P and K. Balanced fertilization with inorganic, organic and biological fertilizers has been suggested to reduce the use of inorganic fertilizers (Rahimi et al., 2019).

Nowadays, biofertilizers utilization by farmers is less intensive than inorganic fertilizers, even though microbial biofertilizers are the key to the nutrients cycle, especially Nitrogen (N) and phosphorus (P) in soil (Bhardwaj et al., 2014). Despite the positive effect of soil beneficial microbes on plant growth, few Indonesian farmers are willing to apply biofertilizers. Coating inorganic NPK fertilizer with organic carrier-based biofertilizers is suggested to intensify the utilization of beneficial microbial by farmers and hence increase the efficiency of using inorganic fertilizers. By using microbial-coated fertilizer, the farmers apply both fertilizers simultaneously, reducing the time spent during crops cultivation.

To achieve those goal, coating NPK with drought-resistance bacteria is proposed since the water content of NPK is as low as 3%. Soil microbes with these characteristics are endospore-forming Bacillus (Toyota, 2015), currently being formulated as commercial biofertilizers. In the soil, several Bacillus species have the natural ability to convert insoluble phosphate (P) into P available for root uptake (Saeid et al., 2018). The Bacillus increase plant growth by phytohormones production. *Bacillus cereus*, *B. megaterium* and *B. subtilis* produce zeatin, zeatin riboside, zeatin glycoside, isopentyl adenine, and isopentyl adenosine (Karadeniz et al., 2006). Secretion of various gibberellins and indole acetic acid by *B. methylotrophicus* KE2 has been reported (Radhakrishnan and Lee, 2016). Moreover, Bacillus tolerates biotic stresses in soil such as salinity and heavy metals (Bal et al., 2013; Syed and Chinthala, 2015) and produce volatile secondary metabolites with antimicrobial or antifungal activity

The Bacillus produce organic acid to provide phosphate (P) through phosphate solubilizing mechanisms (Saeid et al., 2018) and hence increased the yield of paddy (Fitriatin et al., 2021) as well as leafy vegetable crops. Biofertilizers consisting of *B. subtilis, B. pumilu*s, and *B. amyloliquefaciens* is reported to increase the number and weight of lettuce leaves by reducing inorganic fertilizers up to 50% (Venancio et al., 2019). The *B. subtilis* 21-1 increased the yield of Chinese lettuce and decreased soft rot disease by 23.5% and 45%, respectively (Lee et al., 2014). The *B. methylotrophicus* KE2 increasing the height of lettuce plants and improving the amino acids and minerals content of lettuce leaves (Radhakrishnan and Lee, 2016).

Formulation of bacterial inoculant based on drought-resistant Bacillus is recommended for the field with limited-irrigation or in rain-feed agricultural area. The Bacillus forms the endospore in dry condition. In Indonesia, generally farmers cultivate the vegetable in such area. Coating NPK fertilizer with liquid biofertilizer of Bacillus consortium can not only intensify the use of biofertilizers by farmers, but also increase the efficiency use of NPK fertilizer and might decrease the fertilizer dose since Bacillus is able to fix the nitrogen and solubilize the phosphate. In order to develop the BCN, previous study has had two formulas of BCN. However, their effect on crops has not been yet verified. The objective of this experiment was to observed the effect of doses and formulas of bacterial-coated NPK fertilizer BCN and conventional NPK fertilizer on the availability of biomass and the content of N, P and K nutrients in the soil and canopy of lettuce (*Lactuca sativa* L.). Furthermore, this study aimed to observe the potency of BCN fertilizer in substituting some NPK inorganic fertilizers.

## Materials and Methods

The Bacillus-coated NPK (BCN) fertilizer was developed by PT Pupuk Indonesia in collaboration with the Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The pot experiment was carried out in the farmers' area that was covered with ultraviolet plastic; in Mekarwangi Village, Parongpong District, West Bandung Regency, Indonesia, at 1,260 m above sea level. The location was in a tropical mountainous area with a temperature of 17 $^0$C–28 $^0$C.

The pot experiment was conducted on February-April 2021 in Inceptisols with a clay texture (7% sand, 30% silt and 63% clay) and acidity of 6.9. This soil contains 1.85% organic carbon (low), 0.22% total nitrogen (moderate), C/N 8.31 (low), potential $P_2O_5$ 38.31 mg/100 g (moderate), available $P_2O_5$ 14.37 mg/kg (high), potential $K_2O$ 23.57 mg/100 g (moderate). The cation exchange capacity and base saturation of the soil were 34.29 cmol/kg (high) and 49.75% (moderate) respectively. In general, the soil was moderately fertile.

**Experimental Establishment.** The experimental layout was a randomized block design with seven NPK fertilization treatments and five replications. The control treatment was the recommended dosage of NPK fertilizer (16:16:16) for lettuce (200 kg/ha). The seven treatments were A: Control (without NPK fertilizer), B: 1 dose of NPK fertilizer; C: ½ dose of conventional NPK; D: 1 dose of BCN-1; E: ½ dose of BCN-1; F: 1 dose of BCN-2 and G: ½ dose of BCN-2. The dose of BCN was similar to that of NPK.

Two BCN formulas used in this pot experiment were BCN-1 and BCN-2 with 0.2% and 0.4% Bacillus liquid inoculant, respectively, in the coating process. Coated fertilizer contains four Bacillus strains isolated from the rhizosphere of vegetable plants. Each Bacillus isolate was cultured in molasses-based broth for three days at room temperature; liquid inoculant contained $10^9$ colony-forming units (CFU)/mL of Bacillus.

The 17-days old lettuce seedlings were grown in 5 kg potted soil in a 25 x 35 cm polybag. The soil was mixed with manure at a rate of 30 t/ha. The NPK and BCN were applied in split application at 7 and 18 days after transplanting with half dose each. The fertilizer was placed in a 2-cm deep hole at a distance of 5 cm from the stem; then covered with soil. Pesticide Chlorpyrifos was sprayed on the $10^{th}$ day at a concentration of 2 mL/L with a dose of 4 L/ha. The plants were kept in plastic houses and harvested four weeks after planting (WAP).

**Parameters and Statistical Analysis.** Plant height and number of leaves were measured at 3 and 4 WAP while fresh and dry weight of shoot and roots were analyzed at 4 WAP. The fresh weight of shoots and roots was determined by weighing the shoots and roots at harvest while the dry weight was obtained after the plant parts were heated at 60 ˚C to a constant weight. The ratio of shoot to root were calculated based on their dry weight (Ericsson, 1995). Leaf chlorophyll content was determined from 3 fully-opened leaves nearest the growing tip using Soil Plant Analysis Development (SPAD).

The level of N, P and K in the soil as well as in lettuce shoot were measured at 4 WAP by proximate analysis according to the method of the Association of Official Agricultural Chemists (AOAC, 2012). The absorption of N, P and K in the shoot was then calculated by multiplying the nutrient content by the dry weight. The vegetative cell and spore populations of Bacillus in the rhizosphere were counted at 4 WAP by serial dilution plate method on Tryptic Soy Agar. Spores were counted after heating at 80 $^0$C for 15 minutes. All data were analyzed by analysis of variance and continued with Duncan's Multiple Range test at the level of 5%.

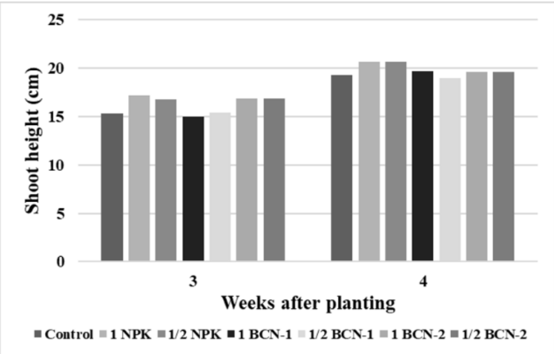
## Results and Discussion

**Plant Growth.** During the experiment, plants grew well in the polybag without any pests and diseases (Figure 1). At the transplanting time, seedlings have 5-cm height and 2 leaves; and at four weeks after planting, the shoot height were approximately 15-17 cm with about 7-9 leaves.

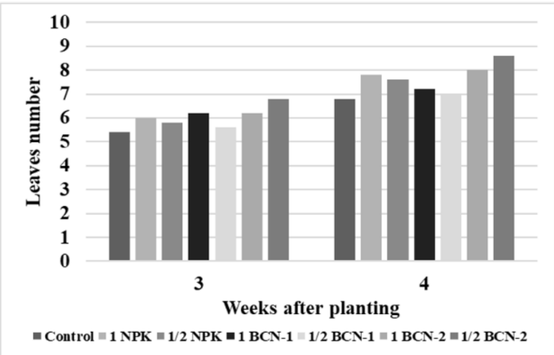
**Figure 1. The 16-days old lettuce grown in potted soil with different dose and formula of Bacillus-coated NPK.**

In current study, the plant height and number of lettuce leaves treated with conventional NPK or coated NPK were not statistically different with the control treatment

(Figure 2). The data verified that the soil nutrients were support the vegetative growth of lettuce. Before experiment, the C/N of soil were low enough (8.31) to mobilize the N for root uptake. Moreover, the available P in soil is high and the total K was moderate. Therefore, NPK as well as BCN application in such soil might be not effective to induce plant growth. Nonetheless, long term leafy vegetable cultivation is considered to reduce the nutrient availability in soil, then the fertilizer application in appropriate dose is suggested.




(a)




(b)

**Figure 2. Shoot weight (a) and leaves number (b) of 3-weeks and 4 weeks-old lettuce after application of bacillus-coated NPK**

The dose and formula of BCN also had no effect on the dry weight of roots and shoots (Table 1). All combination of NPK fertilizer dose and type caused similar growth with control (Fig 2); which is all plant are possibly produce the same quantity of photosynthate in shoot and roots. However, the application of NPK as well as coated NPK have a potency to increase the weight of shoot (edible part of lettuce) up to 24-45 % (Table 1). The used of half dose of NPK as well as BCN resulted in the same yield; which is BCN can be utilized to replace the NPK. Shoot weight with one dose of NPK and half dose of BCN was equal since the Bacillus contribute to the plant growth (Kashyap et al., 2019). Based on previous research, each Bacillus produced the phytohormones IAA, cytokinins and gibberellins which regulate plant growth and development as well as eluviate stress tolerance (Fahad et al., 2015).

**Table 1. Effect of Bacillus-coated NPK on fresh and dry weight of 4-weeks old lettuce**

| Coated NPK treatments | Fresh weight (g) | | Dry weight (g) | |
|---|---|---|---|---|
| | Shoot | Root | Shoot | Root |
| Control | 23.82 a | 2.12 a | 1.22 a | 0.35 a |
| 1 dose NPK | 33.17 a | 2.25 a | 1.59 a | 0.26 a |
| ½ dose NPK | 33.70 a | 2.72 a | 1.45 a | 0.24 a |
| 1 dose BCN-1 | 31.54 a | 2.19 a | 1.49 a | 0.31 a |
| ½ dose BCN-1 | 29.74 a | 1.80 a | 1.38 a | 0.24 a |
| 1 dose BCN-2 | 34.20 a | 2.35 a | 1.61 a | 0.29 a |
| ½ dose BCN-2 | 32.44 a | 2.56 a | 1.41 a | 0.31 a |

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

Application of conventional and coated NPK clearly increased S/R (Table 2) which explained the any dose of NPK fertilizers including bacterial-coated NPK induces shoot growth. The N is a key nutrient in vegetative growth. In plant treated with BCN, shoot growth promotion was interfered by bacterial phytohormones. Auxin is important for cell elongation and cytokinin signaling involve in shoot development in tissue culture as in planta since they promote mitotic cell division of shoot (Schaller et al., 2014). Bacillus might be released available P from inorganic compound by producing organic acids (Saeid et al., 2018). The P play an important role in the synthesis of ATP for providing the energy in metabolism. This is important for tropical soils with high P adsorption capacity.

Surprisingly, plant with half dose of NPK did not show any decrease on biomass and S/R. The recommended dose of NPK is possibly too high for the leaf lettuce. However, this experiment's results align with the potential reduction of urea fertilizer by Bacillus-Azotobacter bacteria-coated urea on strawberry seedlings (Hindersah et al., 2021). Using a slightly different method, incorporating Bacillus bacteria into diammonium phosphate and Urea have a role in efficient use of the two fertilizers. in wheat plants (Ahmad et al., 2017).

Table 2 showed that variations in doses and types of BCN resulted in differences in chlorophyll content index (CCI). Unexpectedly, the CCI of lettuce leaves without any fertilizer is comparable to that of plants with one dose of NPK; and half dose of NPK increased the CCI compared to plants with recommended dose of NPK. the application of ½ dose of BCN-1 and 1 dose of BCN-2 produced leaves with chlorophyll levels that were not different; but higher than the other treatments including control and plants with recommended dose of NPK

**Table 2. Effect of Bacillus-coated NPK on shoot to root ratio (S/R) and chlorophyll content of 4-weeks old lettuce**

| Coated NPK treatments | S/R | Chlorophyll (CCI)* |
|---|---|---|
| Control | 3.49 a | 7.86 b |
| 1 dose NPK | 6.12 b | 8.66 b |
| ½ dose NPK | 6.04 b | 19.20 c |
| 1 dose BCN-1 | 4.81 b | 3.00 a |
| ½ dose BCN-1 | 5.75 b | 12.90 ab |
| 1 dose BCN-2 | 5.55 b | 17.22 bc |
| ½ dose BCN-2 | 4.55 b | 8.94 b |

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %. * CCI: Chlorophyll Content Index

The main nutrient in chlorophyll is nitrogen. The photosynthetic process assimilating $CO_2$ decreases under N deficiency (Prsa et al., 2007). Bacillus has been reported to provide N through $N_2$ fixation (Zakry et al., 2012; Kumar, 2014). In this pot experiment, the increase in the chlorophyll content index can be attributed to the increase in the availability of N in the soil by N fixation, which enzymatically converts $N_2$ into ammonia. Furthermore, ammonia in the soil is reduced to $NH_4^+$ and through the enzymatic nitrification reaction it changes form to $NO_3^-$ (Barth et al., 2020). Nonetheless, this current study did not analyze the available N in soil. The differences response of the plant chlorophyll index to doses and BCN formulas which might be caused by the adaptability of Bacillus in the soil, as well as differences in the effectiveness of N fixation due to different concentration of Bacillus cells on each BCN formula.

**Bacillus Population in Lettuce Rhizosphere.** A recommended dose of NPK, as well as full and half dose of any BCN formula resulted in more Bacillus vegetative cells in rhizosphere compared to plant without fertilizer (Figure 3).

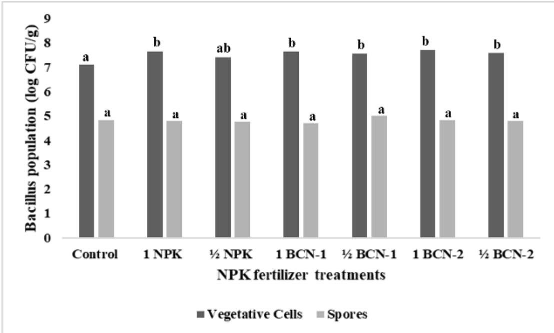



**Figure 3. Population of Bacillus vegetative cells and spores in lettuce rhizosphere at 4-weeks old lettuce grown with BCN fertilizer. Different numbers on each histogram show a significant difference according to Duncan's Multiple Range test at the level of 5%**

Meanwhile, the density of Bacillus spores in all treatments was not significantly different than the control. Vegetative cells were more responsive to fertilization than their spores since the NPK fertilizer is also nutrient source for bacterial cell proliferation. Sporulation from vegetative cells is particularly induced by drought environment and limited oxygen for metabolisms (Toyota, 2015). In this study, soil was always in aerobic and field capacity soil; therefore, the sporulation might not be induced.

The increase of Bacillus count in the rhizosphere might induced by IAA, Cytokinins and Gibberellins produced by Bacillus since all strains enable to synthesize these phytohormone. Auxin is a growth hormone in cell elongation as well as leaves, cambium cells and root development (Zhao et al., 2021). Gibberellins can prevent dwarf plant development (Davière and Achard, 2013) while Cytokinins increase roots cell division in the presence of an auxin (Muraro et al., 2021). In this current experiment, the Bacillus have a significant role to increase the S/R which is plant shoot (Table 1) which is stimulate growth of lettuce.

**Major nutrients in soil and lettuce shoots.** Duncan's test showed that the dose and type of conventional and coated NPK fertilizers did not affect the N and P content but changed the K content in soil (Table 3). At the end of the experiment, the plant soil P levels were moderate-to-high which would become phosphate residue for the next crop. Unexpectedly, the soil K without fertilizer was similar to the K content with full dose of conventional NPK. In general, the soil K content with the application of coated NPK at any dose was same with the soil with the control treatment.

**Table 3. Effect of Bacillus-coated NPK on nitrogen, phosphor and potassium content in potted soil of 4-weeks old lettuce**

| Coated NPK treatments | Nutrient content (%) | | |
|---|---|---|---|
| | N | P | K |
| Control | 0.78 a | 2.98 a | 0.31 b |
| 1 dose NPK | 0.54 a | 2.21 a | 0.30 b |
| ½ dose NPK | 0.46 a | 1.76 a | 0.26 ab |
| 1 dose BCN-1 | 0.77 a | 1.43 a | 0.17 a |
| ½ dose BCN-1 | 0.45 a | 1.86 a | 0.26 ab |
| 1 dose BCN-2 | 0.75 a | 2.26 a | 0.23 ab |
| ½ dose BCN-2 | 0.65 a | 1.60 a | 0.24 ab |

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

The dose and type of NPK had no effect on N and P content and their uptake in shoots but influence K nutrients in the shoot (Table 4 and Table 5).

**Table 4. Effect of Bacillus-coated NPK on nitrogen, phosphor and potassium content of 4-weeks old lettuce**

| Coated NPK treatments | Nutrient content (%) | | |
|---|---|---|---|
| | N | P | K |
| Control | 5.12 a | 1.13 a | 5.14 ab |
| 1 dose NPK | 5.48 a | 1.24 a | 5.19 b |
| ½ dose NPK | 5.50 a | 1.18 a | 5.35 b |
| 1 dose BCN-1 | 5.76 a | 1.35 a | 4.32 a |
| ½ dose BCN-1 | 4.89 a | 1.20 a | 5.06 ab |
| 1 dose BCN-2 | 5.15 a | 1.33 a | 5.10 ab |
| ½ dose BCN-2 | 5.61 a | 1.48 a | 5.13 ab |

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

Bacillus-based BCN had no effect on P uptake because the soil contains moderate total-N and available P. The N fixation process is inhibited by soil available N because nitrogenase which catalyzes N fixation becomes inactive (Oelze, 2000). Meanwhile the soil reaction was neutral (6.9) and the phosphate will be available for plant; in this case the Bacillus might be less involved in phosphate solubilization process. The pattern of increasing K uptake (Table 3) is in line with the soil K content (Table 4). The Bacillus probably released organic acids which were effective in dissolving $K^{2+}$ available from inorganic K (Pramanik et al., 2019).

**Table 5. Effect of Bacillus-coated NPK on nitrogen, phosphor and potassium uptake of 4-weeks old lettuce**

| Coated NPK treatments | Nutrient uptake (mg/plant) | | |
|---|---|---|---|
| | N | P | K |
| Control | 73.73 a | 16.33 a | 74.13 ab |
| 1 dose NPK | 84.12 a | 17.85 a | 85.21 b |
| ½ dose NPK | 79.31 a | 16.99 a | 77.09 ab |
| 1 dose BCN-1 | 83.06 a | 19.52 a | 62.32 a |
| ½ dose BCN-1 | 70.50 a | 17.30 a | 72.98 ab |
| 1 dose BCN-2 | 74.16 a | 19.21 a | 73.46 ab |
| ½ dose BCN-2 | 80.87 a | 21.34 a | 73.92 ab |

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

Current study showed that the levels of N and P in the shoots of lettuce are high but K levels are rather low. According to Jones et al. (1991), the levels of N, P and K of canopy of loose-leaves lettuce are sufficient if they contain 3.50-4.5% of N, 0.45-0.6% of P and 6.60-9.0% of K. This experiment showed that the application of recommended-dose NPK (200 kg/ha) and bacteria-coated NPK with the same dose did not increase N, P and K uptake compared to half the recommended dose (100 kg/ha).

## Conclusion

The dose and formula of Bacillus-coated NPK (BCN) did not affect the height and number of lettuce leaves, biomass, soil N and P content, and N and P uptake of lettuce shoots compared to control and conventional NPK. Both NPK and BCN increased the shoot to root ratio; while only full dose of NPK have increased soil K and K uptake. Any dose and formula of BCN slightly reduce the K in soil and plant compared to NPK treatment. In current study, the population of either vegetative cell or endospore of Bacillus remain similar irrespective of NPK fertilization.

The shoot height and fresh weight of lettuce did not influence by dose and type of NPK which is verified that half dose of any NPK fertilizer produce the comparable yield, Nonetheless, the lettuce has high N and P content but low K. The results explained that recommended dose of NPK can be reduced up to 50% by using conventional NPK or Bacillus-coated NPK. In order to increase K content in plant shoots, additional potassium fertilizer is needed. This experiment showed that conventional or microbial-coated NPK fertilizer can be applied at half dose in Andisol with moderate available N and P, high total P and moderate total K.

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