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## Effectiveness of Bacillus-Based Biofertilizer on Growth and Biomass of Corn in Low-Phosphorus Soil under Pot Experiment

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### ABSTRACT

Biofertilizers provide plants with essential nutrients through nitrogen fixation and phosphate solubilization. A pot experiment was conducted to examine the effects of different types of biofertilizers on plant growth, biomass, and rhizosphere microbial populations in corn grown in soil with low available phosphorus. The greenhouse experiment was designed using a completely randomized block design to test three biofertilizer types and two control treatments. The tested biofertilizers included a solid inoculant of phosphate-solubilizing bacteria, a liquid inoculant of a mixture of nitrogen-fixing bacteria and phosphate-solubilizing bacteria, and a Bacillus-based liquid biofertilizer. The results showed that soil inoculation with biofertilizer in soil enriched with organic matter did not alter leaf number or shoot height in 4-week-old corn; however, it increased both parameters compared to corn grown in soil without cow manure. Despite reduced root growth after biofertilizer inoculation, the corn dry weight was significantly higher following Bacillus-based biofertilizer application than with other biofertilizers. The experiment found that Bacillus was more effective for increasing corn biomass during vegetative growth in soil enriched with organic matter. The results indicate that the effectiveness of heterotrophic biofertilizer microbes is determined by soil organic matter.

Keywords: Bacillus, Biomass, Organic matter, Nitrogen-fixing bacteria, Phosphate-solubilizing Microbes

## Efektivitas Pupuk Hayati Bacillus Pada Pertumbuhan dan Biomasa Jagung Pada Percobaan Pot Dengan Tanah Rendah Fosfor

### ABSTRAK

Pupuk hayati menyediakan nutrisi penting bagi tanaman melalui fiksasi nitrogen dan pelarutan fosfat. Percobaan pot dilakukan untuk menganalisis pengaruh berbagai jenis pupuk hayati terhadap pertumbuhan tanaman, biomassa, dan populasi mikroba di rizosfer jagung yang ditanam di tanah dengan ketersediaan fosfor rendah. Rancangan percobaan rumah kaca adalah rancangan acak kelompok yang menguji tiga jenis biofertilizer dan dua perlakuan kontrol. Pupuk hayati yang diuji meliputi inokulan padat bakteri pelarut fosfat, inokulan cair campuran bakteri pengikat nitrogen dan bakteri pelarut fosfat, dan pupuk hayati cair *Bacillus* spp. Hasil penelitian menunjukkan bahwa inokulasi pupuk hayati pada tanah yang diperkaya pupuk kotoran sapi tidak mempengaruhi jumlah daun dan tinggi tajuk jagung umur 4 minggu; namun, kedua parameter tersebut meningkat dibandingkan dengan jagung yang ditanam di tanah tanpa bahan organik. Pertumbuhan akar berkurang setelah inokulasi pupuk hayati, tetapi berat kering jagung secara signifikan lebih tinggi setelah aplikasi *Bacillus* spp. dibandingkan dengan inokulan lainnya. Percobaan ini memperlihatkan bahwa Bacillus lebih efektif dalam meningkatkan biomassa jagung selama pertumbuhan vegetatif di tanah yang diperkaya dengan bahan organik dibandingkan dengan jenis pupuk hayati lainnya. Hasil percobaan ini mengindikasikan bahwa efektivitas mikroba pupuk hayati heterotrof ditentukan oleh bahan organik tanah.

Kata kunci: Bacillus, Bahan organik, Bakteri pemfiksasi nitrogen, Biomasa, Mikroba pelarut fosfat

## INTRODUCTION

Sustainable agriculture supports the 12th goal of responsible production and consumption within the Sustainable Development Goals. A plant nutrition system in sustainable farming requires an eco-friendly nutrient source to minimize soil damage while maintaining soil health, especially due to the heavy use of inorganic fertilizers.

Biofertilizer can decrease the use of inorganic fertilizers because it supplies essential nutrients like nitrogen (N) and phosphorus (P) through natural nitrogen fixation and phosphate solubilization, respectively. It is widely accepted that free-living N-fixing bacteria (NFB) in the rhizosphere provide plants with available N for root absorption. These bacteria enzymatically convert N<sub>2</sub> to NH<sub>3</sub>, which is then chemically reduced in soil to NH<sub>4</sub><sup>+</sup> and transformed into NO<sub>3</sub><sup>-</sup> by aerobic nitrifying bacteria in both acidic and alkaline conditions. A review stated that free-living NFB provide 10-50 kg N ha<sup>-1</sup> per year [1]. Under controlled conditions, the well-known free-living NFB *Azotobacter* has a N fixation capacity of 8.14-8.46 mg N/g of glucose [2]. The N fixation capacity of *Azospirillum* ranges from 3.3 to 13.11 mg N/g of substrate [3].

Phosphate-solubilizing microbes (PSM) are soil microbes that produce organic acids capable of releasing phosphorus (P) from its insoluble complexes with Al, Fe, Ca, and Mg, thus converting unavailable phosphorus into plant-available forms [4]. In addition to releasing fixed P, this group of microbes can produce phosphatase enzymes. The phosphatase enzymes secreted by these microbes can mineralize organic P into inorganic P [5]. The process of organic P mineralization directly influences plant-available P. Recently, the role of spore-

forming *Bacillus* in nitrogen metabolism has been reported. The six *Bacillus* isolates grow well in Nitrogen-Free Broth, indicating their ability to fix N<sub>2</sub> [6].

Based on laboratory experiments, various strains of NFB and PSM produce phytohormones. *Azotobacter* produces 3.07-459 mg/mL of IAA [7], cytokinin, and gibberellin [8]. *Azospirillum* is a well-known nonsymbiotic NFB that produces IAA through both tryptophan-dependent and -independent pathways [9]. Another potential of PSM is its role as PGPR (plant growth-promoting rhizobacteria), which can produce phytohormones [10].

The impact of NFB on plant growth and yield has been documented. Wheat production can potentially save nearly 25% of its mineral nitrogen requirements by using cyanobacteria or diazotrophic bacteria as inoculants [11]. Mixed inoculation of NFB and PS bacteria was more effective in promoting chili growth than NFB alone [12]. Applying PSM to soil shows positive effects on nutrient levels and crop yields. Using phosphate-solubilizing bacteria and organic fertilizer significantly increased soil P availability, enhanced phosphatase enzyme activity, and improved corn growth in Andisols [13]. Reported increases in corn yields of up to 34% and enhanced plant P uptake were observed after adding phosphate-solubilizing bacteria like *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, which are plant growth-promoting bacteria [14]. However, the comparative effectiveness of various biofertilizer types on corn plants has not been widely studied.

The Faculty of Agriculture at Universitas Padjadjaran develops various biofertilizers, including solid and liquid PSB types and a mix of PDB and N-fixing bacteria, to promote eco-friendly, responsible plant production. Assessing the comparative effects

of these biofertilizers is necessary to determine their effectiveness in enhancing corn growth. Corn productivity in Indonesia needs to be boosted to meet increasing demand for food and feed. This pot experiment aimed to examine plant growth, biomass, and rhizosphere microbial populations in corn grown in low-available phosphorus soil, using different types of NFB and PSM biofertilizers.

## MATERIALS AND METHODS

A pot experiment was conducted in the greenhouse at the Faculty of Agriculture, Universitas Padjadjaran, located in Jatinangor at an elevation of 735 meters above sea level, during the 2024 dry season. The soil was taken from a natural area on the border of food crop plantations and a sand and gravel mine in Rancabawang, Cinanjung Village, Tanjungsari District, Sumedang Regency, West Java. It had a pH of 6.53, contained 2.58% organic carbon, 0.17% total nitrogen, a C/N ratio of 15, 23 mg of potential P<sub>2</sub>O<sub>5</sub> per 100 g, 1.62 mg of available P<sub>2</sub>O<sub>5</sub> per kg, and 18.96 mg of potential K<sub>2</sub>O per 100 g. The soil was low in exchangeable K, Na, and Ca but high in Mg, resulting in a medium cation exchange capacity and low base saturation. The soil texture was silty clay loam, consisting of 11% sand, 56% silt, and 33% clay.

The carrier-based biofertilizer containing P-solubilizer bacteria consortia (BIOP) and the liquid biofertilizer with N-fixer bacteria and P-solubilizer microbes (BION-UP) were developed by the Faculty of Agriculture at Universitas Padjadjaran. PT Pupuk Kujang produced the Bacillus-based liquid biofertilizer in collaboration with the Faculty of Agriculture at Universitas Padjadjaran. The BIOP is composed of the consortium of phosphate-solubilizing bacteria (*Pseudomonas mallei* and *P. cepacea*) and phosphate-solubilizing fungi (*Penicillium* sp. and *Aspergillus* sp.); while BION-UP was

formulated using N-fixer bacteria of *Azotobacter chroococcum*, *A. vinelandii*, *Azospirillum* sp., and *Acinetobacter* sp., along with P-solubilizer *Pseudomonas cepacia*, and *Penicillium* sp. The Bacillus fertilizer is composed of four species of P-solubilizing Bacillus that also produce phytohormones.

## Experimental Design

The pot experiment was set up in a completely randomized block design with five treatments and six replicates. The treatments included three biofertilizer treatments inoculated into the soil and two control treatments, as follows:

- C1: Control (Soil only)
- C2: Soil with organic matter (5:1) without biofertilizer
- B1: C2 + Carrier-based biofertilizer containing P-solubilizing bacteria consortia (BIOP)
- B2: C2 + Liquid biofertilizer with nitrogen-fixing bacteria and phosphorus-solubilizing microbes (BION-UP)
- B3: C2 + liquid inoculant of Bacillus-based consortia biofertilizer (Bacillus)

The liquid biofertilizer dose was 5 L/ha, equivalent to 10 mL per pot, while the solid biofertilizer dose was 5 kg/ha, which is 10 g per pot. All the treatment was replicated six times

## Implementation of Pot Experiment

The pots contained 300 g of a soil mixture, comprising Inceptisols and cow manure in a 5:1 volume ratio. The manure was thoroughly mixed with soil seven days prior to the experiment and stored in the greenhouse until the corn seeds were planted. The Hybrid corn cv. Bisi-2 seeds were soaked in sterilized water for 36 hours to allow the plumule and radicle to emerge. One seedling was transplanted into each pot, to a depth of 2 cm, and covered with soil. Throughout the

experiment, chemical fertilizer was not applied. Biofertilizers were inoculated in split applications at 7 and 14 days after sowing using the soil drenching method. The volume of liquid biofertilizer added to each pot was diluted to 50 mL with water before application. A carrier-based biofertilizer was applied in a 2-cm hole around the plant stems.

Potted corns were maintained in the greenhouse for four weeks and watered daily. During the experiment, no pesticides were used. The number of leaves and plant height were measured weekly from week one to four. At week four, the roots were separated from the shoots, and the rhizosphere of individual roots was collected using a small brush. The root height, along with the fresh and dry weights of the shoot and root, was analyzed. The populations of bacteria and fungi in the rhizosphere were counted using the serial dilution plate method on nutrient agar and potato dextrose agar, respectively.

### Statistical Analysis

All data were analyzed using a one-way analysis of variance (F test,  $p < 0.05$ ) to

assess the effects of the treatments on the tested parameters. When the treatments had a significant impact on a specific parameter, Duncan's Multiple Range Test (DMRT) was performed at  $p < 0.05$ . All statistical analyses were conducted with SPSS version 25.

## RESULTS AND DISCUSSION

### Corn Growth

In the first week, the number of leaves stayed the same with biofertilizer (Table 1). The leaf count of corn grown in the substrate with manure increased by 25% in the second week, regardless of biofertilizer treatments, and this increase reached 34% by the end of the experiment. A similar growth trend was seen in shoot height (Table 2). The shoot height of corn grown in soil without manure or biofertilizer was the lowest. Four weeks after sowing, the manure amendment, with or without biofertilizer, resulted in about a 33% increase in plant growth compared to soil-only treatment. Tables 1 and 2 show that corn growth traits with biofertilizer treatments are similar to those of corn grown in a mix of soil and manure without biofertilizer.

Table 1. Number of corn leaves grown with and without biofertilizer over four weeks

Treatments	Leaf number during 4 weeks			
	1	2	3	4
C1: Only soil	2.8	4.0 a	4.7 a	4.7 a
C2: C1 + manure	3.0	5.0 b	7.0 b	7.0 b
B1: C2 + BIOP	3.0	5.0 b	6.5 b	6.5 b
B2: C2 + BION-UP	3.0	5.0 b	6.7 b	6.7 b
B3: C2 + Bacillus	2.8	4.8 b	6.5 b	6.5 b

Numbers in a column followed by the same letters are not significantly different base on DMRT with  $p < 0.05$

Table 2. Corn shoot height with and without biofertilizer over four weeks

Treatments	Shoot height (cm) during 4 weeks			
	1	2	3	4
C1: Only soil	21.02 a	43.08 a	53.03 a	57.83 a
C2: C1 + manure	22.42 b	48.42 b	73.17 b	79.18 b
B1: C2 + BIOP	22.83 b	49.32 b	72.33 b	77.10 b
B2: C2 + BION-UP	23.50 b	51.75 b	73.00 b	77.28 b
B3: C2 + Bacillus	23.08 b	50.58 b	70.33 b	74.02 b

Numbers in a column followed by the same letters are not significantly different base on DMRT with  $p < 0.05$

In the fourth week, the corn grown in the soil-only treatment had longer roots than those in soil with manure and biofertilizer (Figure 1). The significant decrease in root

length of corn grown with organic matter was up to 18.8% compared to the C1 (soil-only substrate).

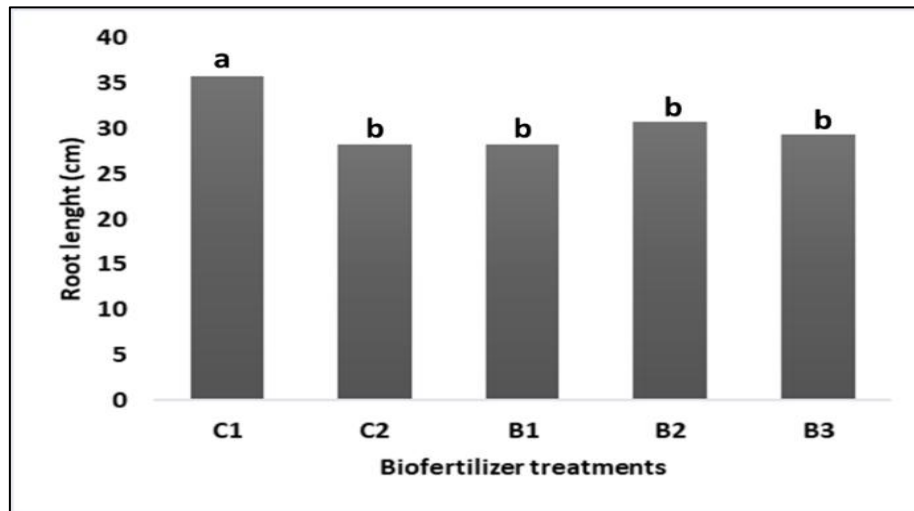


Figure 1. The root height of four-week-old corn grown with and without biofertilizer. C1: Soil only; C2: C1 + manure; B1: C2 + BIOP; B2: C2 + BION-UP; B3: C2 + Bacillus

Organic matter played a key role in boosting growth and biomass despite the soil's average organic carbon content. It is generally accepted that organic matter enhances soil physical properties, mainly porosity and water infiltration [15]. Before the experiment, the soil was classified as silty clay loam, composed of 11% sand, 56% silt, and 33% clay. Adding manure to aerobic clay soil reduces the root barrier, encouraging better root growth. However, the only root trait measured in the experiment was its length, which did not fully reflect the entire root system. Improved physical properties provide more nutrients, although organic matter contains fewer nutrients compared to chemical fertilizers. This benefits plant growth and nutrient uptake.

All microbes in biofertilizers are heterotrophic, relying on organic carbon for energy and growth. However, the

biofertilizer's failure to promote plant growth may result from low soil nutrient levels, especially phosphorus (P) and exchangeable potassium (K). During the experiment, no chemical fertilizers were used, leading to nutrient competition and decreased microbial growth. A pot experiment inoculating liquid cultures of *Azospirillum*, *Azotobacter*, *Bacillus*, and *Lactobacillus*, combined with chemical fertilizer, helps corn grow better [16].

### Plant Biomass

Although the biofertilizer did not improve plant growth parameters, it significantly increased the fresh biomass of both the above- and below-ground parts of corn. Meanwhile, the *Bacillus*-based biofertilizer boosted the fresh weight of the roots and the dry weight of both roots and shoots (Table 3). The *Bacillus* consortia enable plants to produce 162% and 481%

more dry weight in roots and shoots, respectively, compared to plants grown in soil-only substrate. However, the weight

increase in Bacillus-treated corn was approximately 105% compared to manure-enriched soil.

Table 3. Effect of biofertilizers on root and shoot biomass of corn at 4 weeks after sowing

Treatments	Fresh weight (g)		Dry weight (g)	
	Root	Shoot	Root	Shoot
C1: Only soil	4.57a	5.65a	1.63a	0.76a
C2: C1 + manure	7.33c	19.18b	2.12a	4.00b
B1: C2 + BIOP	5.83b	16.50b	2.14a	3.64b
B2: C2 + BION-UP	6.18ab	18.68b	2.28a	3.66b
B3: C2 + Bacillus	9.65d	18.55b	4.34b	4.42c

Numbers in a column followed by the same letters are not significantly different base on DMRT with  $p < 0.05$

Dry weight refers to the total biomass produced through photosynthesis, excluding its water content. Proper nutrient absorption by roots is a crucial factor that influences photosynthesis. Although biofertilizers are generally not very effective at promoting plant growth, Bacillus-based biofertilizers can increase P uptake through P-solubilization mechanisms, thereby improving dry weight. In the previous laboratory experiment, all Bacillus strains in this biofertilizer produced the phytohormones IAA, cytokinin's (zeatin and kinetin), and gibberellins (GA3 and GA4). These metabolites stimulate plant growth; Bacillus may be synthesizing a higher concentration of IAA than cytokinin,

resulting in enhanced root growth [17] for improved nutrient uptake.

### Microbial Population

The total populations of fungi and bacteria in the rhizosphere were approximately 10 and 4, respectively, on a log scale (Figure 2). The fungal population in the corn's rhizosphere decreased significantly by 2.2% after applying Bacillus-based biofertilizer. Meanwhile, bacterial counts in the corn's rhizosphere were unaffected by any biofertilizer. This pot experiment showed that cow manure amendment did not increase both fungal and bacterial populations.

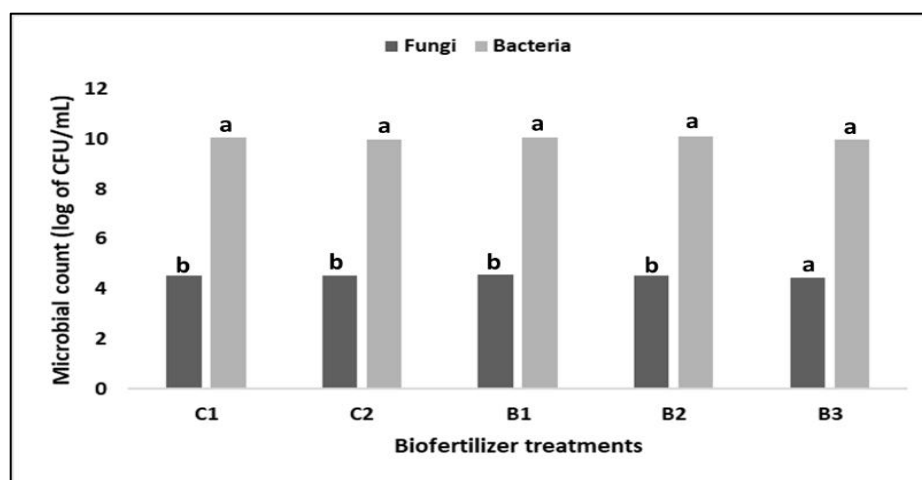


Figure 2. The population of fungi and bacteria in the rhizosphere of 4-week-old corn grown with and without biofertilizer. C1: Only soil; C2: C1 + manure; B1: C2 + BIOP;

## B2: C2 + BION-UP; B3: C2 + Bacillus

An antagonistic effect can occur in the soil between the external *Bacillus* and native fungi, leading to decreased fungal viability. Several *Bacillus* species, including *B. subtilis*, *B. licheniformis*, *B. axarquiensis*, and *Bacillus* sp. isolated from soil, are promising antifungal agents with diverse antifungal activities [18]. The unchanged total bacterial count results from the synergistic effect of all biofertilizers with native bacteria. However, less information is available about the total fungal and bacterial communities to fully understand the precise impact of biofertilizers on beneficial microbes. Monitoring functional microbes and their community structure is necessary.

The lack of effect of biofertilizer on growth suggests that biofertilizer technology does not eliminate agriculture's dependence on chemical fertilizers. Microbes compete with plants for essential nutrients, which were provided solely by the soil in this study. Manure amendments were ineffective at boosting microbial counts in the rhizosphere. In contrast, several studies have reported that inorganic fertilization increases soil microbial counts and alters fungal and bacterial communities in the rhizosphere [19]. The results showed that corn growth in low-N, low-K soils with organic matter and biofertilizer still requires additional nutrients from chemical fertilizers.

Currently, the practical implications of the tested biofertilizers have not been established because their effectiveness remains unclear due to insufficient soil nutrients. More research is needed to assess the benefits of combining inorganic fertilizer with biofertilizer to improve corn growth and yield.

## CONCLUSIONS

Organic matter supported the vegetative growth of corn in soil with an

average organic matter level. However, without macronutrient fertilization (N, P, and K), biofertilizer application alone cannot enhance plant growth or biomass. The *Bacillus*-based liquid inoculant is the only biofertilizer that effectively increases corn dry weight at 4 weeks post-inoculation. None of the biofertilizers affected the bacterial count in the rhizosphere, but the *Bacillus*-based biofertilizer reduced the fungal population. More research is needed to fully understand how corn responds to the combined use of biofertilizer, organic matter, and chemical fertilizers.

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