

Impact of Biological Fertilizers Based on Essential Bacterial Stimulants on Rice Growth and Production

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ABSTRACT

Biological fertilizers are a group of living organisms whose activities can improve soil fertility, fertilizers that contain microbes and are useful for helping plant growth. The purpose of this study was to analyze the growth and production of rice plants against the application of biological fertilizers. There are 7 bacterial isolates used as biofertilizers, namely, *Bacillus* sp., two *Pseudomonas* sp. strains, two *Azospirillum* sp. strains, and two *Azotobacter* sp. strains. This study used an experimental design method through a one-factor randomized block design. The treatment in the study consisted of no fertilization, compost, 100% NPK, 7 isolates enriched compost, 7 isolates enriched compost+50% NPK, 4 isolates enriched compost, and 4 isolates enriched compost+50% NPK. The results showed that compost enriched with biological fertilizers could increase the nutrient content of the soil, thereby increasing the growth and production of rice plants. Treatment of compost enriched with 7 isolates+50% NPK gave the highest yield, both for rice plants. The use of biological fertilizers can reduce the dose of inorganic fertilizer use by up to 50% in rice cultivation.

Keywords: *Azospirillum* sp, *Azotobacter* sp, *Bacillus* sp, biological fertilizers, compost, nutrient, *Pseudomonas* sp, rice growth, rice production.

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I. INTRODUCTION

Biofertilizers are active biological products consisting of microbes that can increase fertilizer efficiency, fertility, and soil health [1]. Bio-fertilizers can fix nitrogen and phosphate solvents which function to increase soil fertility and health, so that plants grow healthier, are free from pests and diseases, nutrient needs are met, yield is higher, sustainable, and can reduce inorganic fertilizers [2].

There are two main roles of biological fertilizers in plant cultivation, namely as a generator of soil life (soil regenerator) and a provider of plant nutrition (Feeding the soil that feeds the plant). microorganisms contained in biological fertilizers require good conditions to grow and develop [3].

[4] explained that the low productivity of rice plants is caused by several factors, including Low pH, the presence of toxic elements Al, Fe, and Mn, and deficiency of nutrients

such as N, P, Ca, and Mg, these conditions are caused by low microbial activity with the number of microbial populations in paddy soil ranging from 29.4.101–14.8.104 cfu gram/land. The microbial population on fertile land is more than 106 cfu gram/soil, this situation indicates that it is time for efforts to increase the fertility of paddy fields by adding essential microbes to biological fertilizers. One of them can utilize biological fertilizers that contain microbes as biological agents.

Plant growth promoting bacteria are Plant Growth Promoting Rhizobacteria, these bacteria are located in the rhizosphere and have the ability to produce phytohormones including *Indole Acetic Acid* (IAA), *cytokinins*, and *gibberellins* [5]. Biofertilizers are a group of living organisms whose activities can improve soil fertility. Application of biological fertilizers based on growth-promoting bacteria from the *Bacillus* sp., *Pseudomonas* sp., *Azospirillum* sp., and

Azotobacter sp, it has been proven to spur rice growth and production [6].

Application of biological fertilizers to rice plants used to enrich compost can increase the number of panicles per hill, the number of grains per hill, the weight of filled grain per hill, and the weight of 1,000 seeds [7]. Research results of [8] concluded that the treatment of biological fertilizers consisting of these isolates was applied to paddy rice and combined with compost and NPK (Nitrogen, Phosphorus, Potassium) at a dose of 50% could increase production compared to the use of NPK (Nitrogen, Phosphorus, Potassium) dose of 100%.

The use of biological fertilizers in paddy fields has not shown significant results, because the fertility of paddy fields is decreasing due to the application of inorganic fertilizers during the rice cultivation process. The low productivity of paddy fields encourages research on the application of biological fertilizers in these fields. This study aims to analyze the growth and production of rice plants by administering essential biological fertilizers with growth stimulant bacteria.

II. METHODOLOGY

This research was conducted on the agricultural land of the North Gorontalo Regency Agriculture Service, Gorontalo Province, Indonesia. From September to December 2022. The material used is rice seeds of the Cakrabuana Variety.

- 1) Compost fertilizer consists of straw and manure (1:1)
- 2) Biofertilizer 1 consists of 7 bacterial isolates: *Bacillus* sp strain DM4, *Pseudomonas* sp strain (PD13 and P3A2), *Azospirillum* sp strain (IDM3 and BGR22), and *Azotobacter* sp strain (23TC and 23TB).
- 3) Biofertilizer 2 consists of 4 bacterial isolates: *Bacillus* sp line DM4, *Pseudomonas* sp line PD13, *Azospirillum* sp strain IDM3, and *Azotobacter* sp 23TC line.
- 4) NPK fertilizers (Nitrogen, Phosphor, Kaliuam) with recommended doses for paddy rice (100% dose) are N (urea 250 Kg/hectare), P (SP-36 100 Kg/hectare) and K (KCl 75 Kg/hectare).

A. Production of Biological Fertilizers

The microbes to be used were first rejuvenated in liquid media and incubated with a shaker for 24 hours for *Bacillus* sp., *Pseudomonas* sp., *Azospirillum* sp., and 48 hours for *Azotobacter* sp. until the cell count reaches 108 cells m/liter. Next, the media containing the bacterial culture was centrifuged for 15 minutes to separate the bacteria from the media liquid to produce a bacterial paste, then as much as 50 ml of the bacterial paste was added to 1 kg of sterile soil.

B. Making Compost Fertilizer

Making compost begins with preparing straw and goat manure with a ratio of 1:1. The straw and goat droppings are arranged in layers, then covered with a tarpaulin. The layers of straw and goat manure were turned over every 10 days. Compost after 2 weeks (half ripe) is enriched with biological fertilizers as much as 2.5% of the initial weight of the compost material. The compost is harvested after 1.5 months.

C. Research Design

The study was designed using a one-factor Randomized Block Design, which consisted of seven fertilization treatments, namely:

P1 = Treatment without fertilization

P2 = NPK Fertilizer Dose 100%

P3 = Compost

P4 = Compost enriched with 7 isolates

P5 = Compost enriched with 7 isolates + NPK dose of 50%

P6 = Compost enriched with 4 isolates

P7 = Compost enriched with 4 isolates + NPK dose of 50%

Each treatment was repeated 3 times so that there were 21 experimental units. One experimental unit was one experimental plot measuring 3×3 m with a paddy spacing of 25×25 cm.

D. Biological Fertilizer Application

Application of microbial-enriched compost is carried out at planting in the planting hole at a dose of 5 tons/hectare (4.5 kg/plot). Application of 100% NPK fertilizer was given at planting time for lowland rice (225 gram/plot urea, 90 g/plot SP-36, and 67.5 g/plot KCl).

E. Soil Analysis

Soil analysis was carried out a month after planting. Soil analysis was carried out for the treatment without fertilization, the addition of 4 isolates of biological fertilizers, and the addition of 7 isolates of biological fertilizers.

F. Growth Parameters

The observed vegetative variables were plant height, number of leaves, and number of tillers every 7 days after planting. The observed outcome component variables are (a) Number of productive tillers, (b) Number of good grain per clump, (c) Weight of 1,000 grain, (d) Percentage of good grain per clump, and (e) yield potential. Yield potential is calculated by the formula: $a \times b \times c \times \text{Plant population of 1 hectare (160,000 plants)}$. Agronomic effectiveness is calculated from production data. The percentage of agronomic effectiveness is calculated by the formula:

$$\frac{\text{Treatment} - \text{Negative Control}}{\text{Positive Control} - \text{Negative Control}} \times 100 \quad (1)$$

Components: negative control (without fertilization) and positive control (100% NPK).

G. Nutrient Uptake Analysis

Analysis of nitrogen and phosphorus content of leaf tissue was carried out before harvest. The nitrogen content of the leaves was analyzed using the *Kjeldahl* method and the phosphorus content in the leaves was analyzed using the spectrophotometer method. The leaves analyzed were mature leaves.

H. Data analysis

Data analysis was carried out using variance at test level $\alpha=5\%$ using the SPSS 16 program. If there is an effect of treatment, it is continued with Duncan's test.

III. RESULTS AND DISCUSSION

A. State of Soil Research Location

Soil analysis was carried out to determine the role of bacterial isolates in biological fertilizers on soil nutrient content. The results of the soil analysis showed that the soil characteristics at the study site had a very acidic soil pH (Table I). Such soil characteristics can be a limiting factor for the growth of rice plants, especially the very acidic pH, low soil nutrient content, and the presence of aluminum (Al). The element Al in the soil fixes P, so that the available P in the soil becomes low.

The low organic C content in the soil is one of the factors for low microbial activity in the soil. The results of soil analysis showed that the microbial isolates in the biological fertilizers added to the compost were able to increase soil nutrients, increase pH and even remove Al elements which are toxic to plants. The nutrient content of Nitrogen (N) and Phosphorus (P) increased after adding compost enriched with bacterial isolates [9]. Bacteria *Azotobacter* sp. and *Azospirillum* sp, has the ability to produce urea reductase which plays an important role in fixing free N from the air, besides that, the genus *Bacillus* sp. and *Pseudomonas* sp. produce phosphatase enzymes which play an important role as P solvents from bound P compounds [10]. Solvent P microbes have the ability to convert insoluble phosphates in the soil into soluble forms by secreting organic acids such as formic, acetic, lactic, sulfuric and propionic acids [11].

The use of compost as an organic material can maintain soil reduction conditions so as to reduce Al in the soil, compost and microbial activity can release organic acids from the decomposition process of organic matter which retains dissolved Al, so that excessive Al availability can be reduced [12]. The organic acids produced by microbes include citric, oxalic, malic, tartaric and malonic acids. These organic acids can reduce the toxicity of Al in paddy soil by binding to Al as a complex compound so that Al is no longer hydrolyzed [13]. Furthermore, the research results of [14] reported that organic acids play an important role in suppressing the solubility of

metal ions by forming helat. Treatment of compost enriched with 7 and 4 bacterial isolates can increase soil pH. [15] explained that an increase in soil pH occurs due to the release of OH⁻ ions resulting from the reduction of organic matter minerals so that the pH in the soil increases. Soil quality in the study area is described in Table I.

B. Components of Rice Plant Growth

The use of compost enriched with 7 isolates added with a dose of 50% NPK (P4) and 4 isolates added with a dose of 50% NPK (P6) significantly increased plant height, number of leaves, and number of tillers of rice compared to the treatment without fertilization and the values were not significantly different from NPK treatment dose of 100% (P2) (Fig. 1). Treatment of compost enriched with microbes can increase the growth parameters of rice plants. The results of this study are in line with the results of the research of [16] which explained that the bacterial consortium consisted of; *Azotobacter* sp, *Azospirillum* sp, *Bacillus* sp, *Pseudomonas* sp, and *Cytophaga* sp contain superior soil microorganisms and are very good for plant growth. According to [17], *Azotobacter* sp bacteria which are aerobic in nature are able to convert nitrogen in the atmosphere into ammonia and then the resulting ammonia is converted into protein needed by plants. *Azospirillum* sp. serves to improve plant productivity through the provision of N₂ or through hormone simulation. *Pseudomonas* sp, and *Bacillus* sp, able to increase nutrient uptake, increase plant growth and productivity.

The compost treatment results in the lowest number of leaves and number of saplings compared to other fertilizer treatments. This is suspected to be a deficiency of Nitrogen nutrients. According to [18] that the nitrogen contained in compost is available slowly for plants because the nature of compost is a slow released fertilizer. Nitrogen nutrients play an important role in the vegetative growth phase of plants. Availability of sufficient Nitrogen nutrients will provide better plant vegetative growth. The growth components of the rice plant are described in Fig. 1.

TABLE I: QUALITY OF PADDY SOIL ONE MONTH AFTER PLANTING

Physical and chemical properties Parameter	Soil Sample		
	P0	P3	P5
pH (H ₂ O)	4.6 (very sour)	4.7 (very sour)	5.3 (a bit sour)
C-Organic (%)	0.77 (very low)	1.67 (low)	1.63(low)
N-total (%)	0.12 (low)	0.23 (moderate)	0.18 low)
C/N ratio	5.8 (low)	8.6 (low)	7.9 (low)
P-available (ppm)	0.5 (very low)	71.8(very high)	28.3 (very high)
K (cmol/kg)	0.24 (low)	17.11 (very high)	7.79(very high)
Al ³⁺ (me 100/gram)	1.16 (very low)	0.12 (very low)	0.24 (very low)

Source: Soil yield data at the Laboratory of the Faculty of Agriculture, Sam Ratulangi University, Manado, Indonesia (2022).

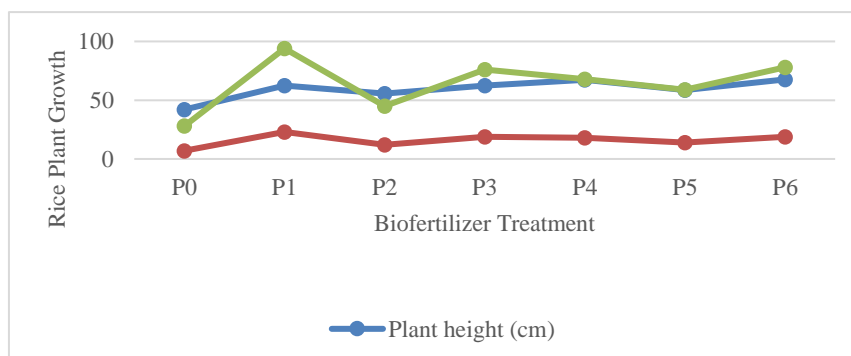


Fig. 1. Components of Rice Plant Growth in All Treatments.

C. Rice Production Components

Provision of microbial-enriched compost can increase production components. This is in accordance with the statement [19] that the increase in the variable component of production is due to an increase in the elements Nitrogen, Phosphorus and Potassium in the soil which are the main nutrients needed by plants. [20] stated that the addition of Nitrogen had a significant effect on the percentage of rice grain. The treatment of compost enriched with microbes gave a weight yield of 1000 grains of rice which was not significantly different from the treatment without fertilization and 100% dose of NPK (Fig. 2). Because the level of soil fertility is still very low, especially the lack of nitrogen elements. Rice plants require high nitrogen, so the role of microbes added to compost to increase nutrients is not optimal. The components of rice crop production through biofertilizer treatment are described in Fig. 2.

The yield potential values obtained showed that the treatment of compost enriched with bacterial isolates was not significantly different from the 100% NPK treatment. [21] stated that inoculation with *Azotobacter* sp. can improve the development of shoots and roots, because these bacteria are able to produce phytohormones for the development of plant crowns and roots. [22] increased rooting caused by division and elongation of root cells stimulated by hormones produced by microbes. [23] added that the application of biological fertilizers containing *Azospirillum* sp. can produce *Indole Acetic Acid* (IAA), which plays a role in the formation and elongation of roots. The production variable is the resultant of vegetative growth. Application of microbial-enriched compost can increase the variable components of rice plant production (Fig. 2). Treatments P3 and P5 were able to increase the number of productive tillers, whereas when combined with NPK 50%, namely treatments P4 and P6, they were able to increase yield potential/hectare.

The hormones produced by these bacteria stimulate the division of root tip cells and lateral roots, thereby creating a favorable root environment. The agronomic effectiveness of the treatment of biological fertilizers combined with inorganic fertilizers gave results that were not significantly different from the 100% dose of NPK treatment. This shows that the treatment of biological fertilizers can reduce the dose of inorganic fertilizers by up to 50% of the recommended dose. The results of this study are in accordance with the research of [24] which concluded that microbes in biological fertilizers can reduce the dose of inorganic fertilizers by up to 50% in food crops. The results of the study of [25] concluded that the inoculation of the bacterium *Azospirillum* sp. In corn crops are able to reduce the need for inorganic fertilizers. Likewise, wheat production increased by 13% in the application of biological fertilizers and NPK fertilizers at a dose of 50% [26].

D. Nutrient Absorption of Biological Fertilizers

The results of leaf nutrient uptake showed that Nitrogen and Phosphorus nutrients absorbed by the leaves in the P2 treatment were higher compared to the others (Table II). The P3 and P5 treatments showed lower nutrient uptake when compared to the P2 treatment. This is because the NPK fertilizer dose of 100% is the recommended fertilizer dose for rice. In addition, NPK fertilizer is a nutrient available for

plants, while compost provides nutrients slowly for plants. Treatments P4 and P6 increased nutrient uptake of Nitrogen and Phosphorus compared to treatments P3 and P5. The results of this study are in line with the results of a study by [27] which explained that applying inorganic fertilizers with biological fertilizers can increase nitrogen and phosphorus uptake in rice plants.

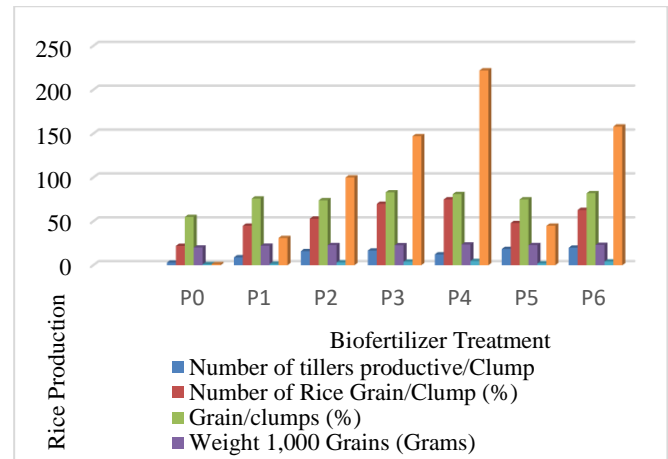


Fig. 2. Components of Rice Production in All Treatments.

High production in rice plants in response to the application of biological fertilizers along with increased nutrient uptake, increased vegetative growth, and increased production variables [28]. This proves that the addition of these isolates to compost can reduce the dose of NPK usage by 50% and the results are even higher than the 100% NPK treatment. The superiority of biological fertilizers to enrich the compost used can improve soil quality, while NPK fertilizer doses of 100% do not improve soil quality. Nutrient absorption of biological fertilizers is described in Table II.

TABLE II: RICE NUTRIENT ABSORPTION

Variety	Treatment	N-content (mg)	P-content (mg)
Cakrabuana	P0	18.99	67.71
	P1	30.82	87.81
	P2	64.08	179.07
	P3	45.93	156.93
	P4	60.11	165.80
	P5	46.98	134.95
	P6	56.76	156.62

Source: Research Result Data after Processing,2022.

Grain weight per plot in compost treatment enriched with 7 bacterial isolates added with 50% NPK fertilizer had the highest production (Fig. 3). Provision of microbial-enriched compost can increase crop yields. [29] stated that the production component is the resultant of the vegetative growth of rice plants. The use of biofertilizers combined with NPK can markedly increase rice crop production. The results of research by [30] concluded that high productivity of rice plants was obtained from the treatment of inorganic fertilizers combined with organic fertilizers. Grain weight per plot is described in Fig. 3.

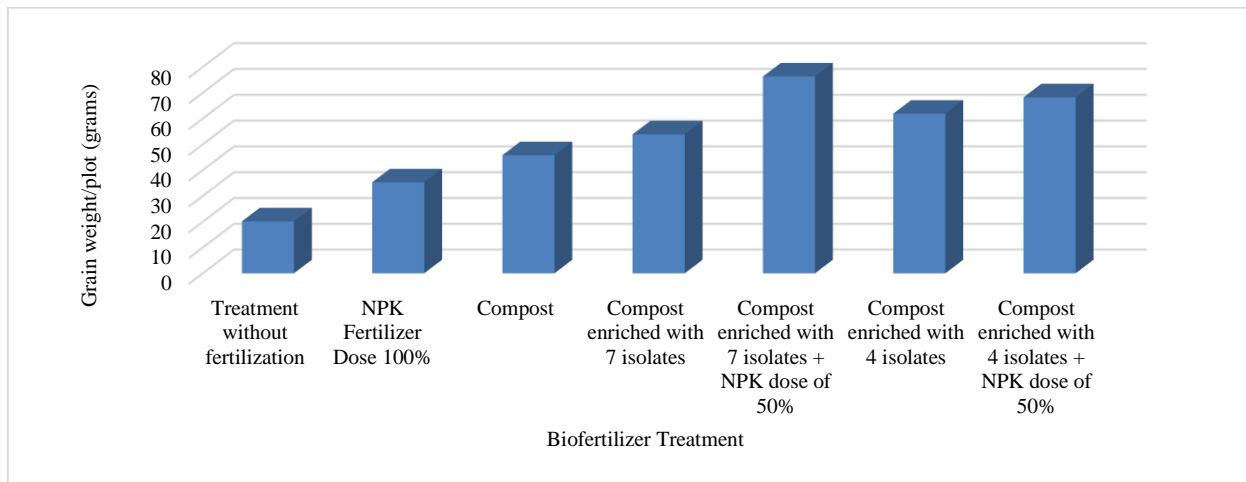


Fig. 3. Production of Dry Unhulled Rice/Plots in All Treatments.

IV. CONCLUSION

Microbial-enriched compost treatment can stimulate the growth and production of Cakrabuana rice varieties. Treatment of compost enriched with 7 microbial isolates and the addition of 50% NPK gave the highest yield. The use of biological fertilizers can reduce the use of NPK fertilizer doses by 50%. The use of microbial enriched compost can improve soil quality in absorbing nutrients.

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CONFLICT OF INTEREST

The authors declare that there is no financial conflict of interest.

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