BUKTI KORESPONDENSI
ARTIKEL JURNAL TERAKREDITASI INTERNASIONAL

Judul Artikel	:	Impact of Biological Fertilizers Based on Essential Bacterial Stimulants on Rice Growth and Production
Jurnal	:	European Journal of Agriculture and Food Sciences (EJFOOD)
ISSN	:	2684-1827
Journal Homepage	:	www.ejfood.org (https://ejfood.org/index.php/ejfood/index)
Volume	:	Vol 5 Issue 2 April 2023
Halaman	:	79 - 84
Article DOI	:	10.24018/ejfood.2023.5.2.648
DOI url	:	http://dx.doi.org/10.24018/ejfood.2023.5.2.648
Penulis	:	Hayatiningsih Gubali Zulzain Ilahude Mohamad Ikbal Bahua Mohamad Lihawa Nikmah Musa Fauzan Zakaria
Corresponding author	:	Mohamad Ikbal Bahua
Email Correspondent	:	mohamad.bahua@ung.ac.id

1. Bukti Konfirmasi Submit Artikel dan Artikel yang di SubmitTanggal 27 Februari 2023

[EJFOOD] Submission Acknowledgement Eksternal Kotak Masuk

Editor-in-Chief <editor@ejfood.org>
kepada saya
27 Feb 2023, 19.43

Dear Author,

Thank you for submitting the manuscript, "Impact of Biological Fertilizers Based on Essential Bacterial Stimulants on Rice Growth and Production" to European Journal of Agriculture and Food Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal website:

Manuscript URL: https://ejfood.org/index.php/ejfood/authorDashboard/submission/648
Username: mohamadikbalbahua

Following steps will be applied for peer-reviewing, copyediting, and publication process after the submission :

a) Peer-Review. We use a double-blind system for peer-review; the reviewers' identities remain anonymous to authors. The paper will be peer-reviewed by two or three experts; one is an editorial staff and the other two are external reviewers. The review process may take 1-2 weeks.

b) Notification of the result of review (Acception, Revision or Decline) by E-mail.

c) The authors make payment for the Article Processing Fee.

d) Copy editing Process-Step 1. All accepted manuscripts are obligated to adapt the layout of the paper according to the journal's template. EJFOOD provides a .DOCX template format.
If authors are unable to adapt the submitted manuscript to the journal's template, the support crew of the EJBIO may provide this service for the authors by charging a Template Support Fee.

e) Copy editing Process-Step 2. After receiving the revised paper, Our copyediting staff will work on the final layout and format. After this process, we will invite you to check the final version of the paper again.

f) Publication. E-journal in .PDF format will be available on the journal's webpage, free of charge for download. In addition, a DOI identifier will be assigned to your paper, and you will be informed regarding the DOI number.

IMPORTANT NOTE 1:

The publisher and journal have a policy of "Zero Tolerance on the Plagiarism". We check the plagiarism issue through two methods: reviewer check and plagiarism prevention tool (iThenticate.com).

All submissions will be checked by iThenticate before being sent to reviewers.

IMPORTANT NOTE 2:

To ensure that you continue getting all the communication emails, please add our email address (editor@ejfood.org) to your contacts. In some cases, the emails sent via our system may end up in your "spam" folder of your personal email account. Therefore, we kindly ask you to periodically check your spam folder during the reviewing and copyediting stages.

If you have any questions, please contact us. Thank you for considering this journal as a venue for your work.

Regards,

EJFOOD Team

Article yang disubmit

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

Hayatiningsih Gubali
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia

Zulzain Ilahude
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia

Mohamad Lihawa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia

Mohamad Ikbal Bahua*
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia

Nikmah Musa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia

Fauzan Zakaria
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia

**Corresponding author.*E-mail:mohamad.bahua@ung.ac.id

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., Pseudomonas sp., compost, rice growth, rice production, biological fertilizers, nutrient

1. INTRODUCTION

Biofertilizers are active biological products consisting of microbes that can increase fertilizer efficiency, fertility and soil health (Sharma *et al*, 2023). 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 (Sayed and Ouis, 2022).

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 (Mahmud *et al*, 2021).

Kulsum *et al* (2023) 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 (Kumar *et al*, 2022).

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* (Dos Santos *et al*, 2022). 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 (Cao *et al*, 2023).

Application of biological fertilizers to rice plants used to enrich compost can increase the number of panicles per hill, the number of grain per hill, the weight of filled grain per hill, and the weight of 1,000 seeds (El-Sobky *et al*, 2022). Research results of Kumar *et al* (2022) 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. MATERIALS AND METOE

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.

- Compost fertilizer consists of: straw and manure (1:1)
- Biofertilizer 1 consisting 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).
- Biofertilizer 2 consisting of 4 bacterial isolates: *Bacillus* sp line DM4, *Pseudomonas* sp line PD13, *Azospirillum* sp strain IDM3, and *Azotobacter* sp 23TC line.
- NPK fertilizers (Nitrogen, Phospor, 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) .

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.

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.

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.

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).

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.

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 × b × c × 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:

$$\frac{Treatment - Negative\ Control}{Positive\ Control - Negative\ Control} x\ 100$$

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

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.

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

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 1). 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 (Zhang *et al*, 2023). Bacteria *Azotobacter* sp. and *Azospirilum* 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 (Arora *et al*, 2022).

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 (Xiao *et al*, 2022). 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 (Das *et al*, 2022). Furthermore, the research results of Gao *et al* (2022) 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. Chen *et al* (2022) 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 1.

TABLE 1. QUALITY OF PADDY SOIL ONE MONTH AFTER PLANTING

Physical and chemical properties Parameter	Soil Sample		
	P0	P3	P5
pH (H2O)	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)

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) (Figure 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 Jafari *et al* (2021) 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 Rasines *et al* (2023), *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 N_2 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 Ladha *et al* (2022) 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 Figure 1.

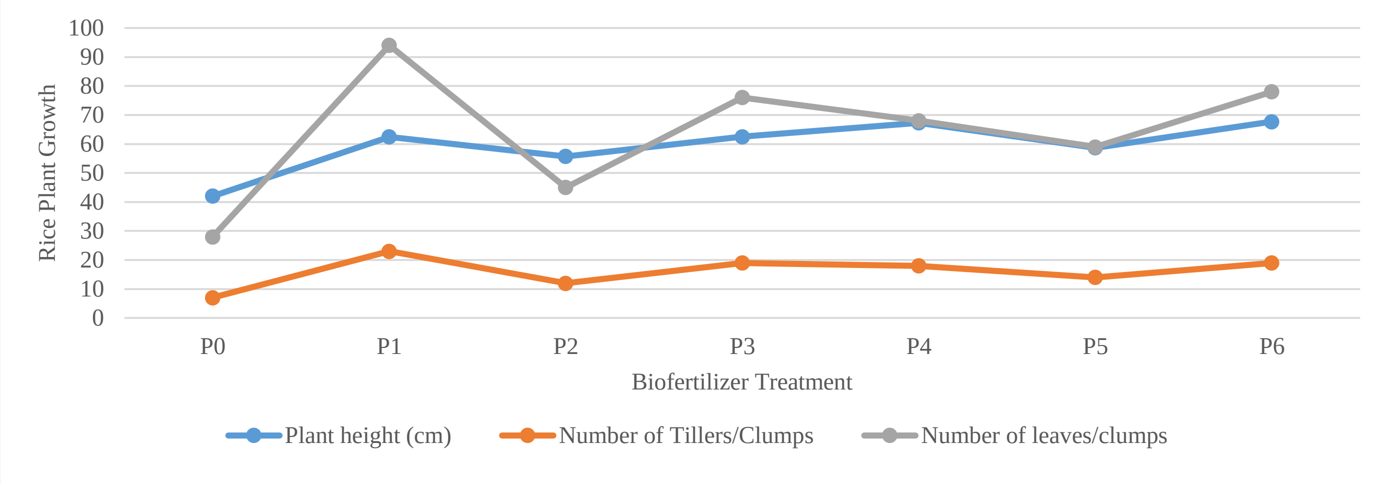


Figure 1. Components of Rice Plant Growth in All Treatments

Rice Production Components

Provision of microbial-enriched compost can increase production components. This is in accordance with the statement of Zhou *et al* (2023) 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. Zhao *et al* (2022) 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 (Figure 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 Figure 2.

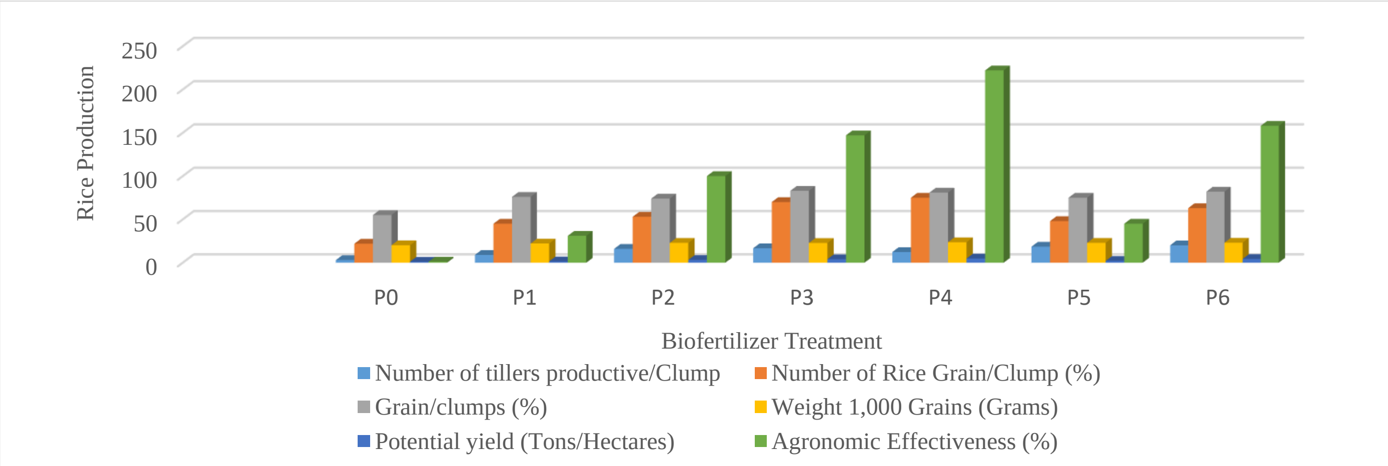


Figure 2. Components of Rice Production in All Treatments

The yield potential values obtained showed that the treatment of compost enriched with bacterial isolates was not significantly different from the 100% NPK treatment. Taheri *et al* (2022) 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. Singh *et al* (2022) increased rooting caused by division and elongation of root cells stimulated by hormones produced by microbes. Emmanuel and Babalola (2020) 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 (Figure 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 up to 50% of the recommended dose. The results of this study are in accordance with the research of Basílio *et al* (2022) 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 Mosqueda *et al* (2022) 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%.

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 2). 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 Exposito *et al* (2022) which explained that applying inorganic fertilizers with biological fertilizers can increase nitrogen and phosphorus uptake in rice plants.

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 (Prabakaran *et al*, 2022). 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 2.

TABLE 2. 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 (Figure 4). Provision of microbial-enriched compost can increase crop yields. Moulick *et al* (2023) 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 Danso *et al* (2023) 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 Figure 4.

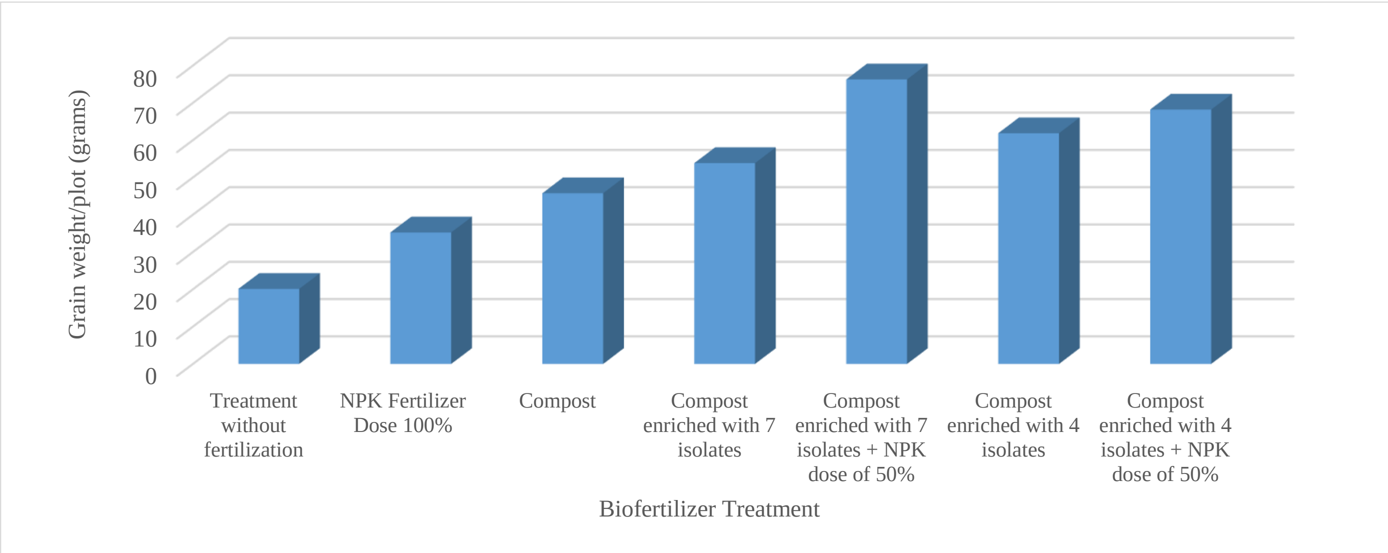


Figure 4. Production of Dry Unhulled Rice/Plots In All Treatments

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.

REFERENCE

Arora, S., Murmu, G., Mukherjee, K., Saha, S. & Maity, D. (2022). A comprehensive overview of nanotechnology in sustainable agriculture. Journal of Biotechnology. 355 (8). 21 - 41.https://doi.org/10.1016/j.jbiotec.2022.06.007.

Basílio, F., Dias, T., Santana, MM, Melo, J., Carvalho, L., Correia, P. & Cruz, C. (2022). Multiple modes of action are needed to unlock soil phosphorus fractions unavailable for plants: The example of bacteria- and fungi-based biofertilizers. Journal of Applied Soil Ecology. 178 (10). 455 - 466.https://doi.org/10.1016/j.apsoil.2022.104550.

Cao, TND, Mukhtar, H., Le, LT, Tran, DPH, Ngo, MTT, Pham, MDT, Nguyen, TB, Quyen Vo, TK & Bui, XT (2023). Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus. Journal Science of The Total Environment. 870(4). 161-174.https://doi.org/10.1016/j.scitotenv.2023.161927.

Chen, K., Liang, J., Xu, X., Zhao, L., Qiu, H., Wang, X. & Cao, X. (2022). Roles of soil active constituents in the degradation of sulfamethoxazole by biochar/persulfate: Contrasting effects of iron minerals and organic matter. Journal Science of The Total Environment. 853 (12). 532 - 548.https://doi.org/10.1016/j.scitotenv.2022.158532.

Danso, F., Agyare, WA & Plange, AB (2023). Benefits and costs of cultivating rice using biochar-inorganic fertilizer combinations. Journal of Agriculture and Food Research. 11(3). 10 - 27.https://doi.org/10.1016/j.jafr.2022.100491.

Das, PP, Singh, KRB, Nagpure, G., Mansoori, A., Singh, RP, Ghazi, IA, Kumar, A. & Singh, J. (2022). Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. Journal of Environmental Research. 214 (11). 821-836.https://doi.org/10.1016/j.envres.2022.113821.

Dos Santos, SRL, Costa, RM, De Aviz, RO, Melo, VMM, Lopes, ACA, Pereira, APA, Mendes, LW, Barbosa, RS & Araujo, ASF (2022). Differential plant growth-promoting rhizobacteria species selection by maize, cowpea, and lima bean. Journal of the Rhizosphere. 24(12). 626-639.https://doi.org/10.1016/j.rhisph.2022.100626.

El-Sobky, ESEA, Taha, AE, El-Sharnouby, M., Sayed, SM & Elrys, USA (2022). Zinc-biochemical co-fertilization improves rice performance and reduces nutrient surplus under semi-arid environmental conditions. Saudi Journal of Biological Sciences. 29(3). 1653 - 1667.https://doi.org/10.1016/j.sjbs.2021.10.066.

Emmanuel, OC & Babalola, OO (2020). Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. Journal of Microbiological Research. 239(10). 656 - 666.https://doi.org/10.1016/j.micres.2020.126569.

Exposito, CDV, Lopez, JA, Liu, J., Bao, N., Liang, J. & Zhang, J. (2022). Development of a cold-active microbial compound biofertilizer on the improvement for rice (Oryza sativa L.) tolerance at low-temperature. Journal of the Rhizosphere. 24(12). 586 - 596.https://doi.org/10.1016/j.rhisph.2022.100586.

Gao. Q., Tao, D., Qi, Z., Liu, Y., Guo, J. & Yu, Y. (2022). Amidoxime functionalized PVDF-based chelating membranes enable synchronous elimination of heavy metals and organic contaminants from wastewater. Journal of Environmental Management. 318 (9). 564 - 578.https://doi.org/10.1016/j.jenvman.2022.115643.

Jafari, F., Khademi, H., Shahrokh, V., Cano, AF, Acosta, JA & Khormali, F. (2021). Biological weathering of phlogopite during enriched vermicomposting. Journal Pedosphere. 31(6). 450-451.https://doi.org/10.1016/S1002-0160(20)60083-2.

Kulsum, PGPS, Khanam, R., Das, S., Nayak, AK, Tack, FMG, Meers, E., Vithanage, M., Shahid, M., Kumar, A., Chakraborty, S., Bhattacharya, T . & Biswas, JK (2023). A state-of-the-art review on cadmium uptake, toxicity, and tolerance in rice: From physiological response to remediation process. Journal of Environmental Research. 220(3). 115 - 127.https://doi.org/10.1016/j.envres.2022.115098.

Kumar, S., Sindhu, SS & Kumar, R. (2022). Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Journal of Current Research in Microbial Sciences. 3(4). 100 - 112.https://doi.org/10.1016/j.crmicr.2021.100094.

Ladha, JK, Peoples, MB, Reddy, PM, Biswas, JC, Bennett, A., Jat, ML & Krupnik, TJ (2022). Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. Journal of Field Crops Research. 283(7). 854 - 868.https://doi.org/10.1016/j.fcr.2022.108541.

Mahmud, K., Missaoui, A., Lee, K., Ghimire, B., Presley, HW & Makaju, S. (2021). Rhizosphere microbiome manipulation for sustainable crop production. Journal of Current Plant Biology. 27(9). 210 - 225.https://doi.org/10.1016/j.cpb.2021.100210.

Marzouk, SH, Tindwa, HJ, Amuri, NA & Semoka, JM (2023). An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. Journal Heliyon. 9(1). 13 - 25.https://doi.org/10.1016/j.heliyon.2023.e13040.

Mosqueda, MCO, Fadiji, AE, Babalola, OO, Glick, BR & Santoyo, G. (2022). Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. Journal of Microbiological Research. 263 (10). 127 - 137.https://doi.org/10.1016/j.micres.2022.127137.

Moulick, D., Ghosh, D., Mandal, J., Bhowmick, S., Mondal, D., Choudhury, S., Santra, SC, Vithanage, M. & Biswas, JK (2023). A cumulative assessment of plant growth stages and selenium supplementation on arsenic and micronutrients accumulation in rice grains. Journal of cleaner production. 386(2). 35 - 47.https://doi.org/10.1016/j.jclepro.2022.135764.

Paravar, A., Piri, R., Balouchi, H. & Ma, Y. (2023). Microbial seed coating: An attractive tool for sustainable agriculture. Journal Biotechnology Reports. 37(3). 781-795.https://doi.org/10.1016/j.btre.2023.e00781.

Prabakaran, S., Mohanraj, T., Arumugam, A. & Sudalai, S. (2022). A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. Journal of Industrial Crops and Products. 183 (9). 942 - 954.https://doi.org/10.1016/j.indcrop.2022.114942.

Rasines, L., Miguel, GS, Garcia, AM, Hernandez, FA, Hontoria, E. & Aguayo, E. (2023). Optimizing the environmental sustainability of alternative post-harvest scenarios for fresh vegetables: A case study in Spain. Journal Science of The Total Environment. 860 (2). 16-30.https://doi.org/10.1016/j.scitotenv.2022.160422.

Sayed, EG & Ouis, MA (2022). Improvement of pea plant growth, yield, and seed quality using glass fertilizers and biofertilizers. Journal of Environmental Technology & Innovation. 26(5). 356 - 368.https://doi.org/10.1016/j.eti.2022.102356.

Sharma, B., Tiwari, S., Kumawat, KC & Cardinale, M. (2023). Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations. Journal Science of The Total Environment. 860 (2). 476-488.https://doi.org/10.1016/j.scitotenv.2022.160476.

Singh, B., Sahu, PM, Aloria, M., Reddy, SS, Prasad, J. & Sharma, RA (2022). Azotobacter chroococcum and Pseudomonas putida enhance pyrroloquinazoline alkaloids accumulation in Adhatoda vasica hairy roots by biotization. Journal of Biotechnology. 353 (7). 51 - 60.https://doi.org/10.1016/j.jbiotec.2022.05.011.

Taheri, E., Tarighi, S. & Taheri, P. (2022). Characterization of root endophytic Paenibacillus polymyxa isolates with biocontrol activity against Xanthomonas translucens and Fusarium graminearum. Journal of Biological Control. 174 (11). 503 - 515.https://doi.org/10.1016/j.biocontrol.2022.105031.

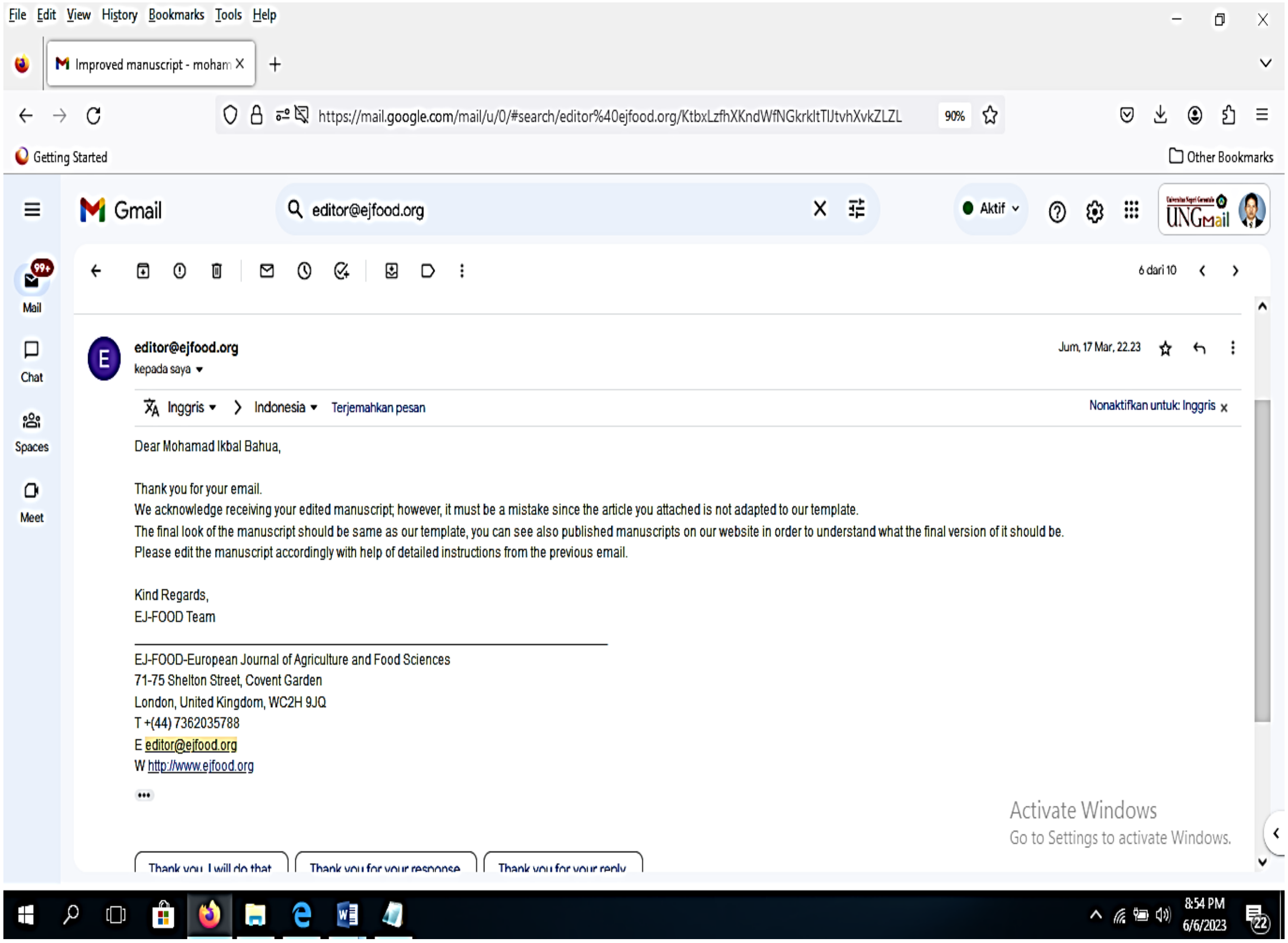
Xiao, J., Wang, G., Liu, H. & Dai, X. (2022). Application of composted lipstatin fermentation residue as organic fertilizer: Temporal changes in soil characteristics and bacterial community. Chemosphere Journal. 306 (11). 135 - 148.https://doi.org/10.1016/j.chemosphere.2022.135637.

Zhang, C., Li, Q., Feng, R., Zhang, Z., Yang, Y. & Liu, J. (2023). C:N:P stoichiometry of plant-soil-microbes in the secondary succession of zokor-made mounds on the Qinghai-Tibet Plateau. Journal of Environmental Research. 222 (4). 115 - 127.https://doi.org/10.1016/j.envres.2023.115333.

Zhao, M., Wu, H., Lu, J., Sun, G. & Du, L. (2022). Effect of grain size on mechanical property and corrosion behavior of a metastable austenitic stainless steel. Journal Materials Characterization. 194 (12). 360-372.https://doi.org/10.1016/j.matchar.2022.112360.

Zhou, L., Xue, J., Xu, Y., Tian, W., Huang, G., Liu, L. & Zhang, Y. (2023). The effect of biochar addition on copper and zinc passivation pathways mediated by humification and microbial community evolution during pig manure composting. Journal of Bioresource Technology. 370(2). 128 - 138.https://doi.org/10.1016/j.biortech.2023.128575.

2. Bukti Konfirmasi Review dan Hasil Review Tanggal 17 Maret 2023


editor@ejfood.org
kepada saya
Jum, 17 Mar, 22.23

Dear Mohamad Ikbal Bahua,

Thank you for your email.
We acknowledge receiving your edited manuscript; however, it must be a mistake since the article you attached is not adapted to our template.
The final look of the manuscript should be same as our template, you can see also published manuscripts on our website in order to understand what the final version of it should be.
Please edit the manuscript accordingly with help of detailed instructions from the previous email.

Kind Regards,
EJ-FOOD Team

EJ-FOOD-European Journal of Agriculture and Food Sciences
71-75 Shelton Street, Covent Garden
London, United Kingdom, WC2H 9JQ
T +(44) 7362035788
E editor@ejfood.org
W http://www.ejfood.org

26

By 26 26

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

Hayatiningsih Gubali
Zulzain Ilahude
Mohamad Lihawa
Mohamad Ikbal Bahua*
Nikmah Musa
Fauzan Zakaria

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., Pseudomonas sp., compost, rice growth, rice production, biological fertilizers, nutrient

1. INTRODUCTION

Biofertilizers are active biological products consisting of microbes that can increase fertilizer efficiency, fertility and soil health (Sharma *et al*, 2023). 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 (Sayed and Ouis, 2022).

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 (Mahmud *et al*, 2021).

Kulsum *et al* (2023) 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 (Kumar *et al*, 2022).

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* (Dos Santos *et al*, 2022). 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 (Cao *et al*, 2023).

Application of biological fertilizers to rice plants used to enrich compost can increase the number of panicles per hill, the number of grain per hill, the weight of filled grain per hill, and the weight of 1,000 seeds (El-Sobky *et al*, 2022). Research results of Kumar *et al* (2022) 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. MATERIALS AND METOE

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.

- Compost fertilizer consists of: straw and manure (1:1)

- Biofertilizer 1 consisting 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).
- Biofertilizer 2 consisting of 4 bacterial isolates: *Bacillus* sp line DM4, *Pseudomonas* sp line PD13, *Azospirillum* sp strain IDM3, and *Azotobacter* sp 23TC line.
- NPK fertilizers (Nitrogen, Phospor, 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) .

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.

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.

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.

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).

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.

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 × b × c × 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:

$$\frac{Treatment - Negative\ Control}{Positive\ Control - Negative\ Control}\, x\ 100$$

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

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.

Data analysis

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

III. RESULTS AND DISCUSSION

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 1). 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 (Zhang *et al*, 2023). Bacteria *Azotobacter* sp. and *Azospirilum* 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 (Arora *et al*, 2022).

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 (Xiao *et al*, 2022). 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 (Das *et al*, 2022). Furthermore, the research results of Gao *et al* (2022) 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. Chen *et al* (2022) 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 1.

TABLE 1. QUALITY OF PADDY SOIL ONE MONTH AFTER PLANTING

Physical and chemical properties Parameter	Soil Sample		
	P0	P3	P5
pH (H2O)	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)

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) (Figure 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 Jafari *et al* (2021) 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 Rasines *et al* (2023), *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 N_2 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 Ladha *et al* (2022) 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 Figure 1.

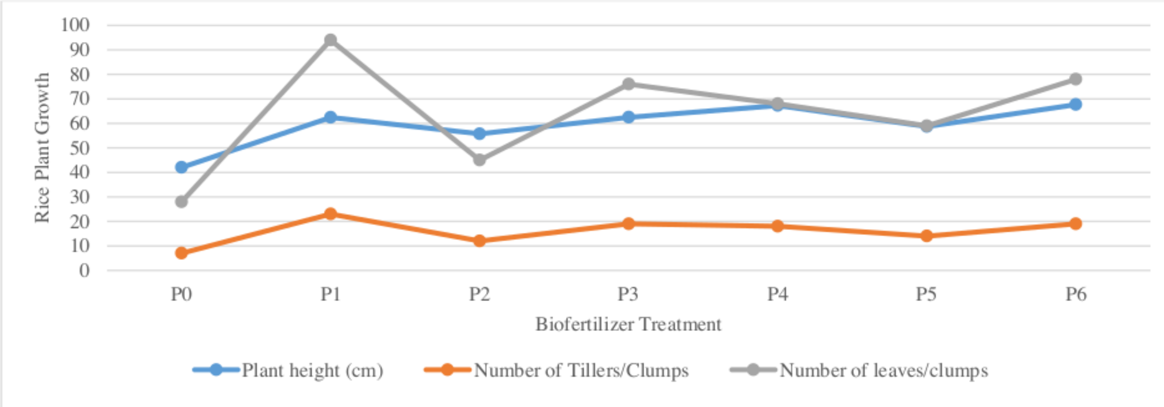


Figure 1. Components of Rice Plant Growth in All Treatments

Rice Production Components

Provision of microbial-enriched compost can increase production components. This is in accordance with the statement of Zhou *et al* (2023) 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. Zhao *et al* (2022) 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 (Figure 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 Figure 2.

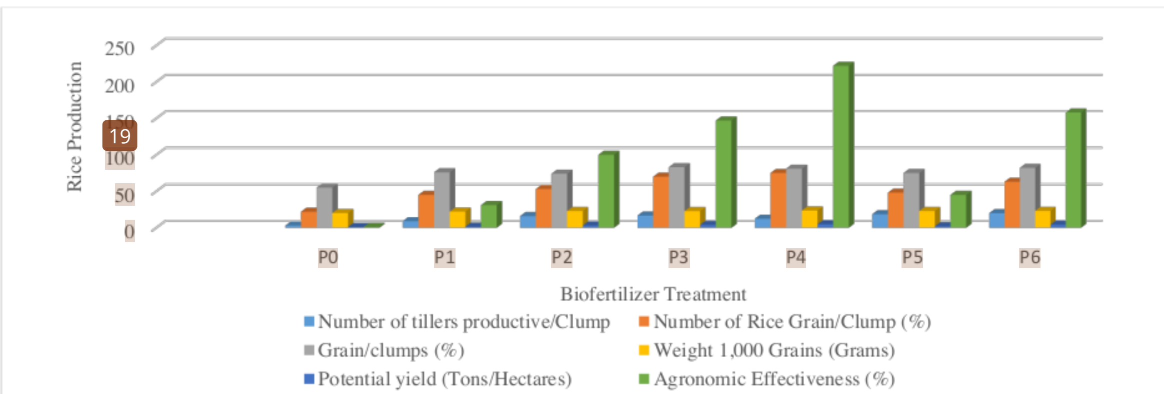


Figure 2. Components of Rice Production in All Treatments

The yield potential values obtained showed that the treatment of compost enriched with bacterial isolates was not significantly different from the 100% NPK treatment. Taheri *et al* (2022) 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. Singh *et al* (2022) increased rooting caused by division and elongation of root cells stimulated by hormones produced by microbes. Emmanuel and Babalola (2020) 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 (Figure 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 up to 50% of the recommended dose. The results of this study are in accordance with the research of Basílio *et al* (2022) 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 Mosqueda *et al* (2022) 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%.

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 2). 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 Exposito *et al* (2022) which explained that applying inorganic fertilizers with biological fertilizers can increase nitrogen and phosphorus uptake in rice plants.

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 (Prabakaran *et al*, 2022). 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 2.

TABLE 2. 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 (Figure 4). Provision of microbial-enriched compost can increase crop yields. Moulick *et al* (2023) 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 Danso *et al* (2023) 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 Figure 4.

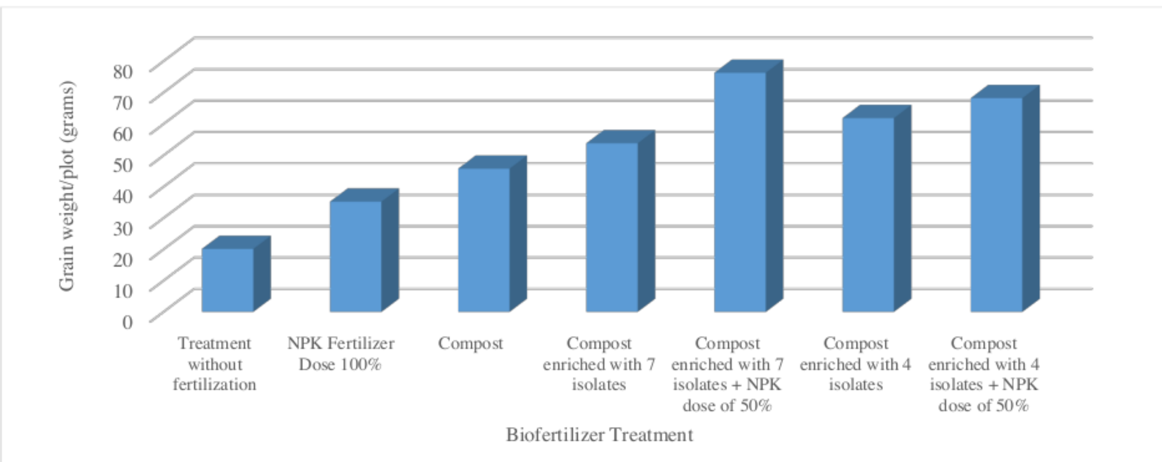


Figure 4. Production of Dry Unhulled Rice/Plots In All Treatments

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.

26

ORIGINALITY REPORT

16%
SIMILARITY INDEX

PRIMARY SOURCES

1	nanopdf.com Internet	102 words — 3%
2	Gergonius Fallo, Nisa Rachmania Mubarik, Triadiati .. "Potency of Auxin Producing and Phosphate Solubilizing Bacteria from Dryland in Rice Paddy Field", Research Journal of Microbiology, 2015 Crossref	53 words — 2%
3	worldwidescience.org Internet	39 words — 1%
4	www.scilit.net Internet	39 words — 1%
5	e-journal.unipma.ac.id Internet	36 words — 1%
6	Izhar Khairullah, Didik Indradewa, Azwar Maas, Prapto Yudono. " The Effect of Straw and Purun Tikus ( ) Compost to Rice Physiological Traits, Iron Toxicity and Grain Yield on Acid Sulfate Soils ", IOP Conference Series: Earth and Environmental Science, 2020 Crossref	33 words — 1%
7	talenta.usu.ac.id Internet	26 words — 1%

8	AR Gusta, M. Same. "The Effect of Organic Fertilizer and NPK on the Growth of the Master Pepper Plants", IOP Conference Series: Earth and Environmental Science, 2022 Crossref	25 words — 1%
9	www.internationaljournalssrg.org Internet	16 words — < 1%
10	D R Ichsanti, S A Aziz, D Efendi. "Rhipsalis baccifera cuttings growth with different cutting segment numbers and planting media", IOP Conference Series: Earth and Environmental Science, 2023 Crossref	14 words — < 1%
11	Maritina Stavrakaki, Despoina Bouza, Katerina Biniari. "Differentiation of Greek grapevine cultivars (Vitis vinifera L.) based on the combination of ampelographic description and microsatellite markers", Genetic Resources and Crop Evolution, 2019 Crossref	14 words — < 1%
12	aip.scitation.org Internet	14 words — < 1%
13	journal.perbanas.ac.id Internet	14 words — < 1%
14	"Plant-Microbe Interactions in Agro-Ecological Perspectives", Springer Science and Business Media LLC, 2017 Crossref	13 words — < 1%
15	"Proceedings of the VIII. Alps-Adria Scientific Workshop, 27 April–2 May 2009, Neum, Bosnia-Herzegovina — CROP PRODUCTION AND PLANT PROTECTION", Cereal Research Communications, 2009	12 words — < 1%

Crossref

16 Wendy Mateo, Hanwu Lei, Elmar Villota, Moriko Qian et al. "Synthesis and characterization of sulfonated activated carbon as a catalyst for bio-jet fuel production from biomass and waste plastics", Bioresource Technology, 2020
Crossref
12 words — < 1%

17 ojs.unud.ac.id
Internet
12 words — < 1%

18 Jingxin Zhang, Yaobin Zhang, Xie Quan, Yang Li, Shuo Chen, Huimin Zhao, Dong Wang. "An anaerobic reactor packed with a pair of Fe-graphite plate electrodes for bioaugmentation of azo dye wastewater treatment", Biochemical Engineering Journal, 2012
Crossref
10 words — < 1%

19 text-id.123dok.com
Internet
10 words — < 1%

20 Soil Biology, 2015.
Crossref
9 words — < 1%

21 www.iiste.org
Internet
9 words — < 1%

22 abstracts.boku.ac.at
Internet
8 words — < 1%

23 journal.uinsgd.ac.id
Internet
8 words — < 1%

24 Irkham Widiyono. "Ovarian Inactivity in Traditionally Raised Indonesian Bligon Goats:
6 words — < 1%

Association with Microminerals", Research Square Platform LLC, 2022
Crossref Posted Content

EXCLUDE QUOTES	OFF	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	OFF	EXCLUDE MATCHES	OFF

Impact of Biological Fertilizers Based on Essential Bacterial Stimulants on Rice Growth and Production (Customized with templates

Hayatiningsih Gubali
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(hayatiningsihgubali@ung.ac.id)

Zulzain Ilahude
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(zulzainilahude@ung.ac.id)

Mohamad Lihawa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(mohamd.lihawa@ung.ac.id)

Mohamad Ikbal Bahua*
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(mohamad.bahua@ung.ac.id)

Nikmah Musa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(nikmahmusa@ung.ac.id)

Fauzan Zakaria
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(fauzanzakaria@ung.ac.id)

*Corresponding author.*E-mail:mohamad.bahua@ung.ac.id

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., Pseudomonas sp., compost, rice growth, rice production, biological fertilizers, nutrient (Latin name of bacteria in italics)

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 grain 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.

- Compost fertilizer consists of: straw and manure (1:1)
- Biofertilizer 1 consisting 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).
- Biofertilizer 2 consisting of 4 bacterial isolates: *Bacillus* sp line DM4, *Pseudomonas* sp line PD13, *Azospirillum* sp strain IDM3, and *Azotobacter* sp 23TC line.
- NPK fertilizers (Nitrogen, Phospor, 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). (Written sequentially with numbers)

Production of Biological Fertilizers (In italics)

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.

Making Compost Fertilizer (In italics)

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.

Research Design (In italics)

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.

Biological Fertilizer Application (In italics)

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).

Soil Analysis (In italics)

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.

Growth Parameters (In italics)

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 × b × c × 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:

$$\frac{Treatment - Negative\ Control}{Positive\ Control - Negative\ Control} x\ 100$$

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

Nutrient Uptake Analysis (In italics)

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.

Data analysis (In italics)

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

III. RESULTS AND DISCUSSION

State of Soil Research Location (In italics)

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 1). 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 *Azospirilum* 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 1.

TABLE 1. QUALITY OF PADDY SOIL ONE MONTH AFTER PLANTING (In written roman numerals)

Physical and chemical properties Parameter	Soil Sample		
	P0	P3	P5
pH (H2O)	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)

Components of Rice Plant Growth (In italics)

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) (Figure 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 N_2 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 Figure 1.

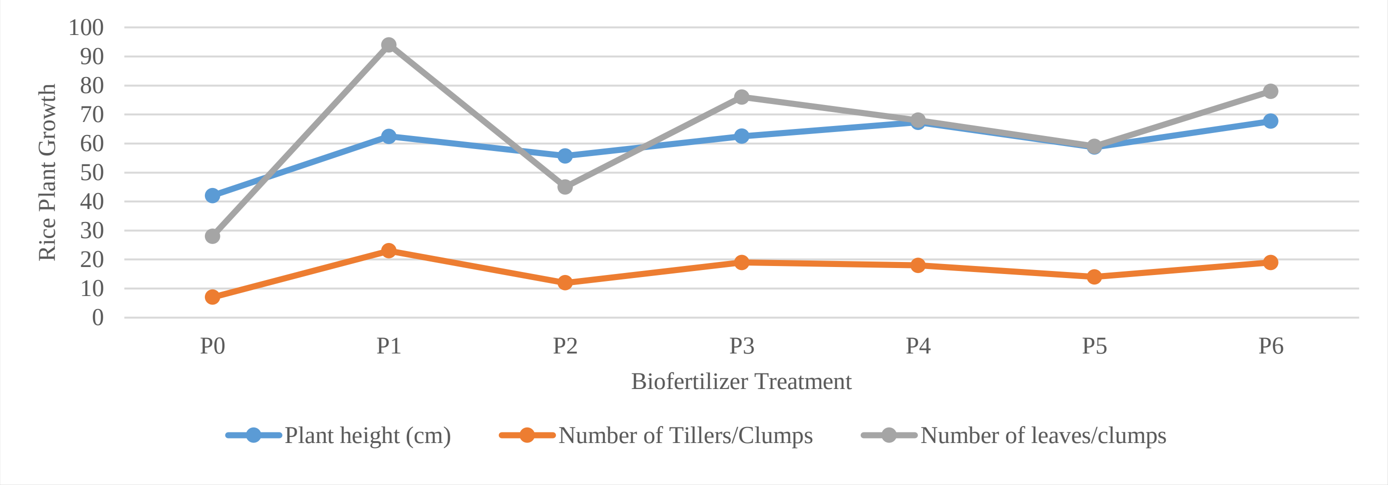


Figure 1. Components of Rice Plant Growth in All Treatments (Write fig and match the layout with the journal template)

Rice Production Components (In italics)

Provision of microbial-enriched compost can increase production components. This is in accordance with the statement of [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 (Figure 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 Figure 2.

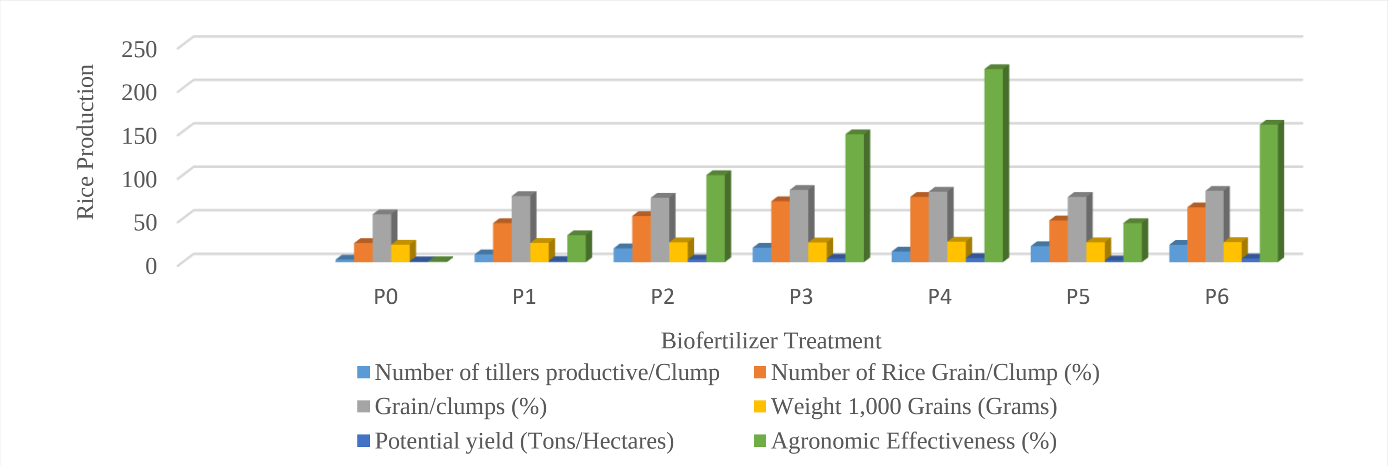


Figure 2. Components of Rice Production in All Treatments (Write fig and match the layout with the journal template)

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 (Figure 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 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].

Nutrient Absorption of Biological Fertilizers (In italics)

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 2). 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.

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 2.

TABLE 2. RICE NUTRIENT ABSORPTION (In written roman numerals)

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 (Figure 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 Figure 3.

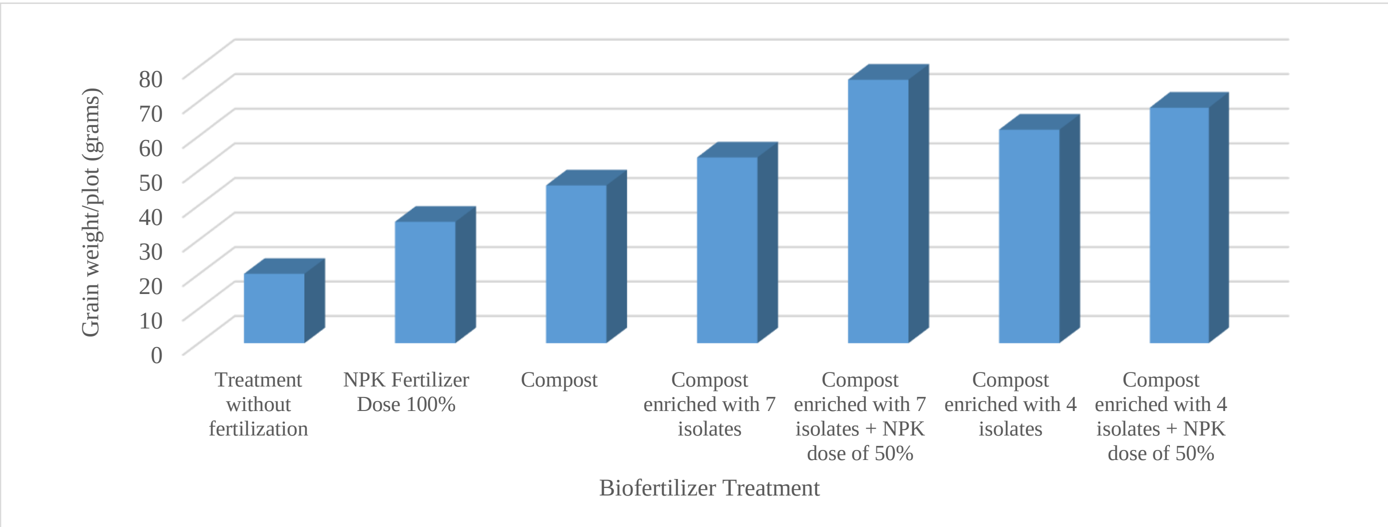


Figure 3. Production of Dry Unhulled Rice/Plots In All Treatments (Write fig and match the layout with the journal template)

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.

Add before the reference:
Acknowledgment
Funding
Conflict of interest

V. REFERENCE

[1] Sharma, B., Tiwari, S., Kumawat, KC & Cardinale, M. Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations. *Journal Science of The Total Environment*. 2023. 860 (2). 476 - 488. https://doi.org/10.1016/j.scitotenv.2022.160476.

[2] Sayed, EG & Ouis, MA. Improvement of pea plant growth, yield, and seed quality using glass fertilizers and biofertilizers. *Journal of Environmental Technology & Innovation*. 2022. 26(5). 356 - 368. https://doi.org/10.1016/j.eti.2022.102356.

[3] Mahmud, K., Missaoui, A., Lee, K., Ghimire, B., Presley, HW & Makaju, S. Rhizosphere microbiome manipulation for sustainable crop production. *Journal of Current Plant Biology*. 2021. 27 (9). 210 - 225. https://doi.org/10.1016/j.cpb.2021.100210.

[4] Kulsum, PGPS, Khanam, R., Das, S., Nayak, AK, Tack, FMG, Meers, E., Vithanage, M., Shahid, M., Kumar, A., Chakraborty, S., Bhattacharya, T . & Biswas, JK. A state-of-the-art review on cadmium uptake, toxicity, and tolerance in rice: From physiological response to remediation process. *Journal of Environmental Research*. 2023. 220 (3). 115 - 127. https://doi.org/10.1016/j.envres.2022.115098.

[5] Dos Santos, SRL, Costa, RM, De Aviz, RO, Melo, VMM, Lopes, ACA, Pereira, APA, Mendes, LW, Barbosa, RS & Araujo, ASF. Differential plant growth-promoting rhizobacteria species selection by maize, cowpea, and lima bean. *Journal of the Rhizosphere*. 2022. 24 (12). 626 - 639. https://doi.org/10.1016/j.rhisph.2022.100626.

[6] Cao, TND, Mukhtar, H., Le, LT, Tran, DPH, Ngo, MTT, Pham, MDT, Nguyen, TB, Quyen Vo, TK & Bui, XT. Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus. *Journal Science of The Total Environment*. 2023. 870 (4). 161 - 174. https://doi.org/10.1016/j.scitotenv.2023.161927.

[7] El-Sobky, ESEA, Taha, AE, El-Sharnouby, M., Sayed, SM & Elrys, USA. Zinc-biochemical co-fertilization improves rice performance and reduces nutrient surplus under semi-arid environmental conditions. *Saudi Journal of Biological Sciences*. 2022. 29 (3). 1653 - 1667. https://doi.org/10.1016/j.sjbs.2021.10.066.

[8] Kumar, S., Sindhu, SS & Kumar, R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Journal of Current Research in Microbial Sciences*. 2022. 3 (4). 100 – 112. https://doi.org/10.1016/j.crmicr.2021.100094.

[9] Zhang, C., Li, Q., Feng, R., Zhang, Z., Yang, Y. & Liu, J. C:N:P stoichiometry of plant-soil-microbes in the secondary succession of zokor-made mounds on the Qinghai-Tibet Plateau. *Journal of Environmental Research*. 2023. 222 (4). 115 - 127. https://doi.org/10.1016/j.envres.2023.115333.

[10] Arora, S., Murmu, G., Mukherjee, K., Saha, S. & Maity, D. A comprehensive overview of nanotechnology in sustainable agriculture. *Journal of Biotechnology*. 2022. 355 (8). 21 - 41.https://doi.org/10.1016/j.jbiotec.2022.06.007.

[11] Paravar, A., Piri, R., Balouchi, H. & Ma, Y. Microbial seed coating: An attractive tool for sustainable agriculture. *Journal Biotechnology Reports*. 2023. 37(3). 781-795. https://doi.org/10.1016/j.btre.2023.e00781.

[12] Xiao, J., Wang, G., Liu, H. & Dai, X. Application of composted lipstatin fermentation residue as organic fertilizer: Temporal changes in soil characteristics and bacterial community. *Chemosphere Journal*. 2022. 306 (11). 135 - 148. https://doi.org/10.1016/j.chemosphere.2022.135637.

[13] Das, PP, Singh, KRB, Nagpure, G., Mansoori, A., Singh, RP, Ghazi, IA, Kumar, A. & Singh, J.. Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Journal of Environmental Research*. 2022. 214 (11). 821 - 836.https://doi.org/10.1016/j.envres.2022.113821.

[14] Gao. Q., Tao, D., Qi, Z., Liu, Y., Guo, J. & Yu, Y. Amidoxime functionalized PVDF-based chelating membranes enable synchronous elimination of heavy metals and organic contaminants from wastewater. *Journal of Environmental Management*. 2022. 318 (9). 564 - 578. https://doi.org/10.1016/j.jenvman.2022.115643.

[15] Chen, K., Liang, J., Xu, X., Zhao, L., Qiu, H., Wang, X. & Cao, X. Roles of soil active constituents in the degradation of sulfamethoxazole by biochar/persulfate: Contrasting effects of iron minerals and organic matter. *Journal Science of The Total Environment*. 2022. 853 (12). 532 - 548. https://doi.org/10.1016/j.scitotenv.2022.158532.

[16] Jafari, F., Khademi, H., Shahrokh, V., Cano, AF, Acosta, JA & Khormali, F. Biological weathering of phlogopite during enriched vermicomposting. *Journal Pedosphere*. 2021. 31 (6). 450 - 451. https://doi.org/10.1016/S1002-0160(20)60083-2.

[17] Rasines, L., Miguel, GS, Garcia, AM, Hernandez, FA, Hontoria, E. & Aguayo, E. Optimizing the environmental sustainability of alternative post-harvest scenarios for fresh vegetables: A case study in Spain. *Journal Science of The Total Environment.* 2023. 860 (2). 16 - 30. https://doi.org/10.1016/j.scitotenv.2022.160422.

[18] Ladha, JK, Peoples, MB, Reddy, PM, Biswas, JC, Bennett, A., Jat, ML & Krupnik, TJ. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Journal of Field Crops Research.* 2022. 283 (7). 854 - 868. https://doi.org/10.1016/j.fcr.2022.108541.

[19] Zhou, L., Xue, J., Xu, Y., Tian, W., Huang, G., Liu, L. & Zhang, Y. The effect of biochar addition on copper and zinc passivation pathways mediated by humification and microbial community evolution during pig manure composting. *Journal of Bioresource Technology.* 2023. 370 (2). 128 - 138. https://doi.org/10.1016/j.biortech.2023.128575.

[20] Zhao, M., Wu, H., Lu, J., Sun, G. & Du, L. Effect of grain size on mechanical property and corrosion behavior of a metastable austenitic stainless steel. *Journal Materials Characterization.* 2022. 194 (12). 360 - 372. https://doi.org/10.1016/j.matchar.2022.112360.

[21] Taheri, E., Tarighi, S. & Taheri, P. Characterization of root endophytic Paenibacillus polymyxa isolates with biocontrol activity against Xanthomonas translucens and Fusarium graminearum. *Journal of Biological Control*. 2022. 174 (11). 503 - 515. https://doi.org/10.1016/j.biocontrol.2022.105031.

[22] Singh, B., Sahu, PM, Aloria, M., Reddy, SS, Prasad, J. & Sharma, RA. Azotobacter chroococcum and Pseudomonas putida enhance pyrroloquinazoline alkaloids accumulation in Adhatoda vasica hairy roots by biotization. *Journal of Biotechnology.* 2022. 353 (7). 51 - 60. https://doi.org/10.1016/j.jbiotec.2022.05.011.

[23] Emmanuel, OC & Babalola, OO. Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. *Journal of Microbiological Research*. 2020. 239 (10). 656 - 666. https://doi.org/10.1016/j.micres.2020.126569.

[24] Basílio, F., Dias, T., Santana, MM, Melo, J., Carvalho, L., Correia, P. & Cruz, C. Multiple modes of action are needed to unlock soil phosphorus fractions unavailable for plants: The example of bacteria- and fungi-based biofertilizers. *Journal of Applied Soil Ecology*. 2022. 178 (10). 455 - 466. https://doi.org/10.1016/j.apsoil.2022.104550.

[25] Mosqueda, MCO, Fadiji, AE, Babalola, OO, Glick, BR & Santoyo, G. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. *Journal of Microbiological Research*. 2022. 263 (10). 127 - 137. https://doi.org/10.1016/j.micres.2022.127137.

[26] Marzouk, SH, Tindwa, HJ, Amuri, NA & Semoka, JM. An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. *Journal Heliyon*. 2023. 9 (1). 13 - 25. https://doi.org/10.1016/j.heliyon.2023.e13040.

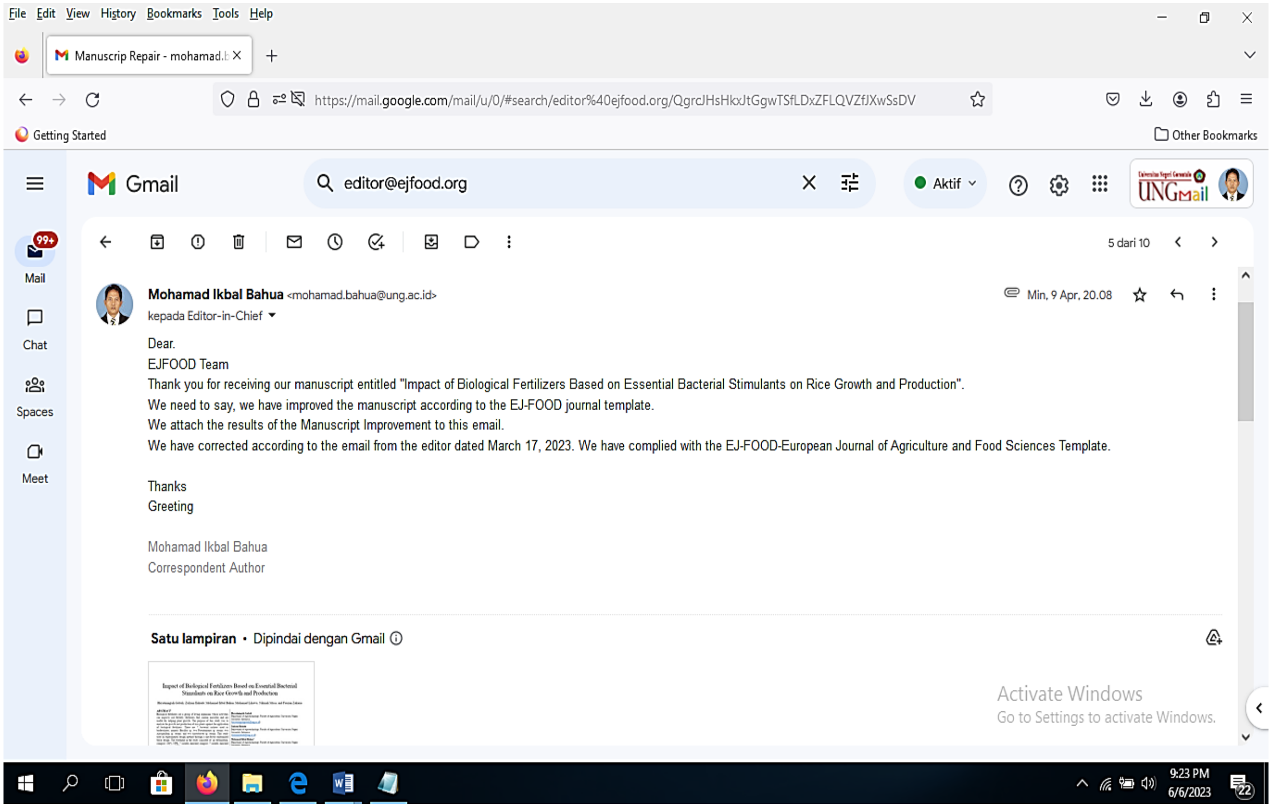
[27] Exposito, CDV, Lopez, JA, Liu, J., Bao, N., Liang, J. & Zhang, J. Development of a cold-active microbial compound biofertilizer on the improvement for rice (Oryza sativa L.) tolerance at low-temperature. *Journal of the Rhizosphere*. 2022. 24 (12). 586 - 596. https://doi.org/10.1016/j.rhisph.2022.100586.

[28] Prabakaran, S., Mohanraj, T., Arumugam, A. & Sudalai, S. A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Journal of Industrial Crops and Products*. 2022. 183 (9). 942 - 954. https://doi.org/10.1016/j.indcrop.2022.114942.

[29] Moulick, D., Ghosh, D., Mandal, J., Bhowmick, S., Mondal, D., Choudhury, S., Santra, SC, Vithanage, M. & Biswas, JK. A cumulative assessment of plant growth stages and selenium supplementation on arsenic and micronutrients accumulation in rice grains. *Journal of Cleaner Production*. 2023. 386 (2). 35 - 47. https://doi.org/10.1016/j.jclepro.2022.135764.

[30] Danso, F., Agyare, WA & Plange, AB. Benefits and costs of cultivating rice using biochar-inorganic fertilizer combinations. *Journal of Agriculture and Food Research*. 2023. 11 (3). 10 - 27. https://doi.org/10.1016/j.jafr.2022.100491.

3. Bukti Konfirmasi Submit Revisi Artikel, Respon kepada Reviewer, dan Artikel Resubmit Tanggal 9 April 2023



European Journal of Agriculture and Food Sciences
www.ejfood.org

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

Hayatiningsih Gubali, Zulzain Ilahude, Mohamad I. Bahua, Mohamad Lihawa, Nikmah Musa, and Fauzan Zakaria

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.

Submitted:

Published:

ISSN: 2684-1827

DOI:

H. Gubali
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: hayatiningsihgubali@ung.ac.id)

Z. Ilahude
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: zulzainilahude@ung.ac.id)

M. I. Bahua*
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: mohamad.bahua@ung.ac.id)

M. Lihawa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: mohamd.lihawa@ung.ac.id)

N. Musa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: nikmahmusa@ung.ac.id)

F. Zakaria
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: fauzanzakaria@ung.ac.id)

Corresponding author

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, Phospor, 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×b×c×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:

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

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 α=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 *Azospirilum* 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 N_2 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	Soil Sample		
Parameter	P0	P3	P5
pH (H2O)	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).

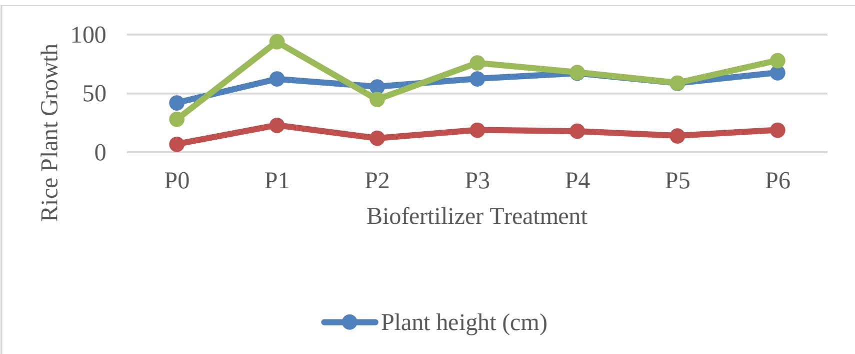


Fig. 1. Components of Rice Plant Growth in All Treatments.

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.

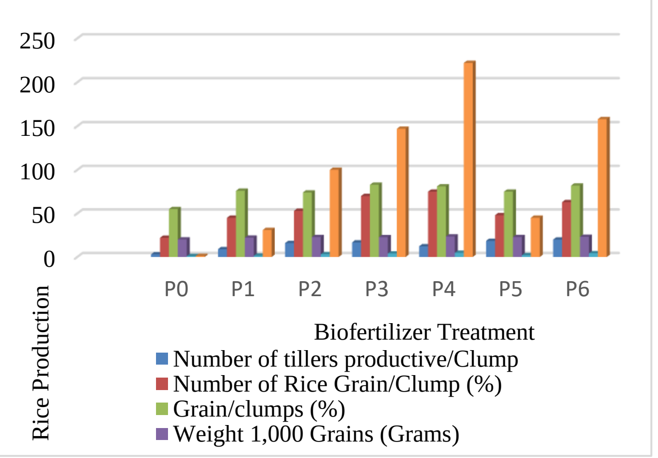


Fig. 2. Components of Rice Production in All Treatments.

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)
	P0	18.99	67.71
	P1	30.82	87.81
	P2	64.08	179.07
Cakrabuana	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.

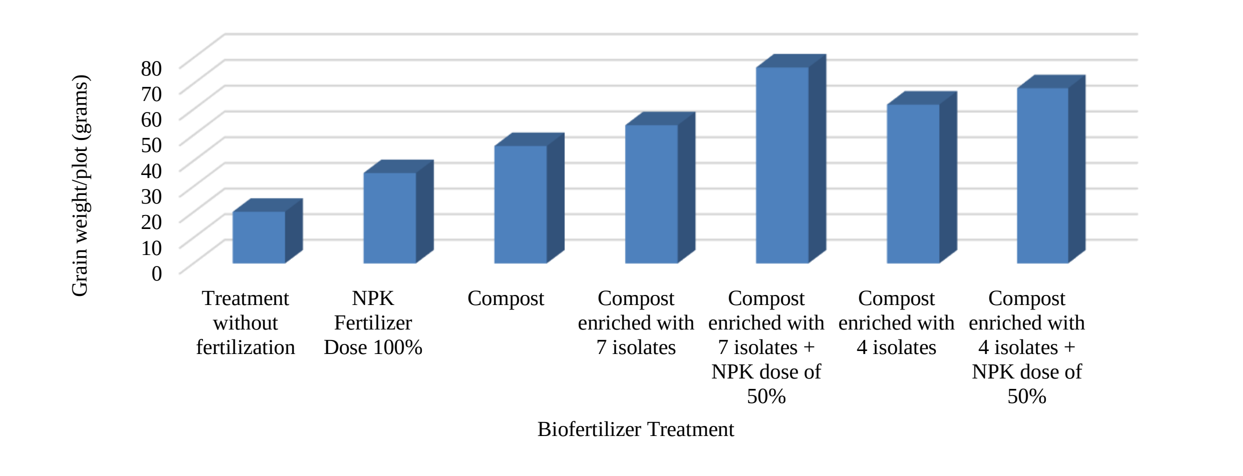


Fig. 3. Production of Dry Unhulled Rice/Plots in All Treatments.

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.

ACKNOWLEDGMENT

The research would like to thank the Government of North Gorontalo District, Gorontalo Province, Indonesia, for allowing us to conduct research on the Agricultural Office Land.

FUNDING

Thank you to the Head of the Department of Agrotechnology, Faculty of Agriculture, Gorontalo State University for funding this research.

CONFLICT OF INTEREST

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

REFERENCES

[1] Sharma, B., Tiwari, S., Kumawat, KC & Cardinale, M. Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations. *Journal Science of The Total Environment*. 2023; 860 (2). 476–488. https://doi.org/10.1016/j.scitotenv.2022.160476.

[2] Sayed, EG & Ouis, MA. Improvement of pea plant growth, yield, and seed quality using glass fertilizers and biofertilizers. *Journal of Environmental Technology & Innovation*. 2022; 26(5). 356–368. https://doi.org/10.1016/j.eti.2022.102356.

[3] Mahmud, K., Missaoui, A., Lee, K., Ghimire, B., Presley, HW & Makaju, S. Rhizosphere microbiome manipulation for sustainable crop production. *Journal of Current Plant Biology*. 2021; 27 (9). 210–225. https://doi.org/10.1016/j.cpb.2021.100210.

[4] Kulsum, PGPS, Khanam, R., Das, S., Nayak, AK, Tack, FMG, Meers, E., Vithanage, M., Shahid, M., Kumar, A., Chakraborty, S., Bhattacharya, T. & Biswas, JK. A state-of-the-art review on cadmium uptake, toxicity, and tolerance in rice: From physiological response to remediation process. *Journal of Environmental Research*. 2023; 220 (3). 115–127. https://doi.org/10.1016/j.envres.2022.115098.

[5] Dos Santos, SRL, Costa, RM, De Aviz, RO, Melo, VMM, Lopes, ACA, Pereira, APA, Mendes, LW, Barbosa, RS & Araujo, ASF. Differential plant growth-promoting rhizobacteria species selection by maize, cowpea, and lima bean. *Journal of the Rhizosphere*. 2022; 24 (12). 626–639. https://doi.org/10.1016/j.rhisph.2022.100626.

[6] Cao, TND, Mukhtar, H., Le, LT, Tran, DPH, Ngo, MTT, Pham, MDT, Nguyen, TB, Quyen Vo, TK & Bui, XT. Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus. *Journal Science of The Total Environment*. 2023; 870 (4). 161–174. https://doi.org/10.1016/j.scitotenv.2023.161927.

[7] El-Sobky, ESEA, Taha, AE, El-Sharnouby, M., Sayed, SM & Elrys, USA. Zinc-biochemical co-fertilization improves rice performance and reduces nutrient surplus under semi-arid environmental conditions. *Saudi Journal of Biological Sciences*. 2022; 29 (3). 1653–1667. https://doi.org/10.1016/j.sjbs.2021.10.066.

[8] Kumar, S., Sindhu, SS & Kumar, R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Journal of Current Research in Microbial Sciences*. 2022; 3 (4). 100–112. https://doi.org/10.1016/j.crmicr.2021.100094.

[9] Zhang, C., Li, Q., Feng, R., Zhang, Z., Yang, Y. & Liu, J. C: N:P stoichiometry of plant-soil-microbes in the secondary succession of zokor-made mounds on the Qinghai-Tibet Plateau. *Journal of Environmental Research*. 2023. 222 (4). 115–127. https://doi.org/10.1016/j.envres.2023.115333.

[10] Arora, S., Murmu, G., Mukherjee, K., Saha, S. & Maity, D. A comprehensive overview of nanotechnology in sustainable agriculture. *Journal of Biotechnology*. 2022; 355 (8). 21–41.https://doi.org/10.1016/j.jbiotec.2022.06.007.

[11] Paravar, A., Piri, R., Balouchi, H. & Ma, Y. Microbial seed coating: An attractive tool for sustainable agriculture. *Journal Biotechnology Reports*. 2023; 37(3). 781–795. https://doi.org/10.1016/j.btre.2023.e00781.

[12] Xiao, J., Wang, G., Liu, H. & Dai, X. Application of composted lipstatin fermentation residue as organic fertilizer: Temporal changes in soil characteristics and bacterial community. *Chemosphere Journal*. 2022; 306 (11). 135–148. https://doi.org/10.1016/j.chemosphere.2022.135637.

[13] Das, PP, Singh, KRB, Nagpure, G., Mansoori, A., Singh, RP, Ghazi, IA, Kumar, A. & Singh, J.. Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Journal of Environmental Research*. 2022; 214 (11). 821–836.https://doi.org/10.1016/j.envres.2022.113821.

[14] Gao. Q., Tao, D., Qi, Z., Liu, Y., Guo, J. & Yu, Y. Amidoxime functionalized PVDF-based chelating membranes enable synchronous elimination of heavy metals and organic contaminants from wastewater. *Journal of Environmental Management*. 2022; 318 (9). 564–578. https://doi.org/10.1016/j.jenvman.2022.115643.

[15] Chen, K., Liang, J., Xu, X., Zhao, L., Qiu, H., Wang, X. & Cao, X. Roles of soil active constituents in the degradation of sulfamethoxazole by biochar/persulfate: Contrasting effects of iron minerals and organic matter. *Journal Science of The Total Environment*. 2022; 853 (12). 532–548. https://doi.org/10.1016/j.scitotenv.2022.158532.

[16] Jafari, F., Khademi, H., Shahrokh, V., Cano, AF, Acosta, JA & Khormali, F. Biological weathering of phlogopite during enriched vermicomposting. *Journal Pedosphere*. 2021; 31 (6). 450–451. https://doi.org/10.1016/S1002-0160(20)60083-2.

[17] Rasines, L., Miguel, GS, Garcia, AM, Hernandez, FA, Hontoria, E. & Aguayo, E. Optimizing the environmental sustainability of alternative post-harvest scenarios for fresh vegetables: A case study in Spain. *Journal Science of The Total Environment.* 2023; 860 (2). 16–30. https://doi.org/10.1016/j.scitotenv.2022.160422.

[18] Ladha, JK, Peoples, MB, Reddy, PM, Biswas, JC, Bennett, A., Jat, ML & Krupnik, TJ. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Journal of Field Crops Research.* 2022; 283 (7). 854–868. https://doi.org/10.1016/j.fcr.2022.108541.

[19] Zhou, L., Xue, J., Xu, Y., Tian, W., Huang, G., Liu, L. & Zhang, Y. The effect of biochar addition on copper and zinc passivation pathways mediated by humification and microbial community evolution during pig manure composting. *Journal of Bioresource Technology.* 2023; 370 (2). 128–138. https://doi.org/10.1016/j.biortech.2023.128575.

[20] Zhao, M., Wu, H., Lu, J., Sun, G. & Du, L. Effect of grain size on mechanical property and corrosion behavior of a metastable austenitic stainless steel. *Journal Materials Characterization.* 2022; 194 (12). 360–372. https://doi.org/10.1016/j.matchar.2022.112360.

[21] Taheri, E., Tarighi, S. & Taheri, P. Characterization of root endophytic Paenibacillus polymyxa isolates with biocontrol activity against Xanthomonas translucens and Fusarium graminearum. *Journal of Biological Control*. 2022; 174 (11). 503–515. https://doi.org/10.1016/j.biocontrol.2022.105031.

[22] Singh, B., Sahu, PM, Aloria, M., Reddy, SS, Prasad, J. & Sharma, RA. Azotobacter chroococcum and Pseudomonas putida enhance pyrroloquinazoline alkaloids accumulation in Adhatoda vasica hairy roots by biotization. *Journal of Biotechnology.* 2022; 353 (7). 51–60. https://doi.org/10.1016/j.jbiotec.2022.05.011.

[23] Emmanuel, OC & Babalola, OO. Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. *Journal of Microbiological Research*. 2020; 239 (10). 656–666. https://doi.org/10.1016/j.micres.2020.126569.

[24] Basílio, F., Dias, T., Santana, MM, Melo, J., Carvalho, L., Correia, P. & Cruz, C. Multiple modes of action are needed to unlock soil phosphorus fractions unavailable for plants: The example of bacteria- and fungi-based biofertilizers. *Journal of Applied Soil Ecology*. 2022; 178 (10). 455–466. https://doi.org/10.1016/j.apsoil.2022.104550.

[25] Mosqueda, MCO, Fadiji, AE, Babalola, OO, Glick, BR & Santoyo, G. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. *Journal of Microbiological Research*. 2022; 263 (10). 127–137. https://doi.org/10.1016/j.micres.2022.127137.

[26] Marzouk, SH, Tindwa, HJ, Amuri, NA & Semoka, JM. An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. *Journal Heliyon*. 2023; 9 (1). 13–25. https://doi.org/10.1016/j.heliyon.2023.e13040.

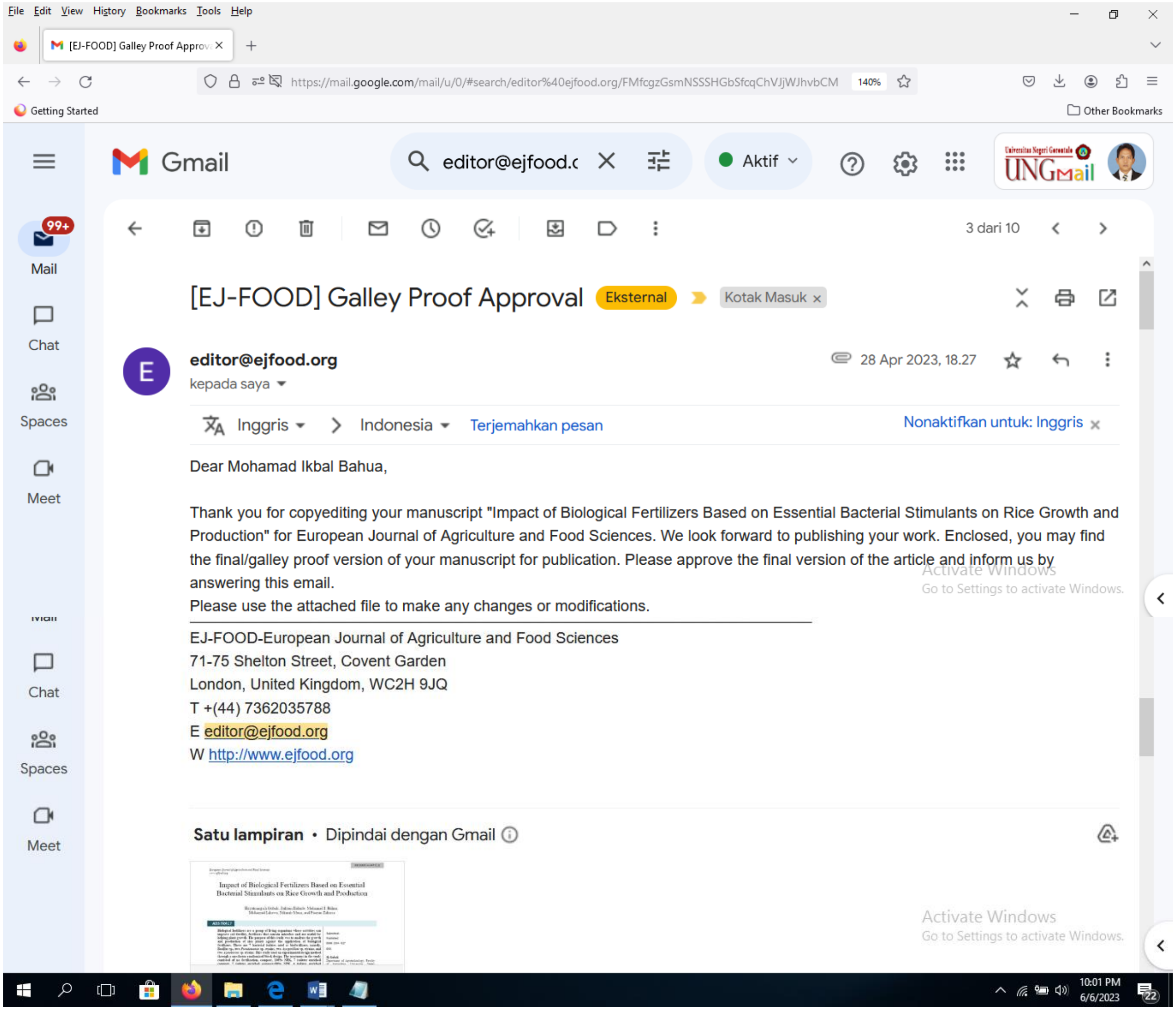
[27] Exposito, CDV, Lopez, JA, Liu, J., Bao, N., Liang, J. & Zhang, J. Development of a cold-active microbial compound biofertilizer on the improvement for rice (Oryza sativa L.) tolerance at low-temperature. *Journal of the Rhizosphere*. 2022; 24 (12). 586–596. https://doi.org/10.1016/j.rhisph.2022.100586.

[28] Prabakaran, S., Mohanraj, T., Arumugam, A. & Sudalai, S. A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Journal of Industrial Crops and Products*. 2022; 183 (9). 942–954. https://doi.org/10.1016/j.indcrop.2022.114942.

[29] Moulick, D., Ghosh, D., Mandal, J., Bhowmick, S., Mondal, D., Choudhury, S., Santra, SC, Vithanage, M. & Biswas, JK. A cumulative assessment of plant growth stages and selenium supplementation on arsenic and micronutrients accumulation in rice grains. *Journal of Cleaner Production*. 2023; 386 (2). 35–47. https://doi.org/10.1016/j.jclepro.2022.135764.

[30] Danso, F., Agyare, WA & Plange, AB. Benefits and costs of cultivating rice using biochar-inorganic fertilizer combinations. *Journal of Agriculture and Food Research*. 2023; 11 (3). 10–27. https://doi.org/10.1016/j.jafr.2022.100491.

4. Bukti Konfirmasi Artikel Accepted Tanggal 28 April 2023



European Journal of Agriculture and Food Sciences
www.ejfood.org

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

Hayatiningsih Gubali, Zulzain Ilahude, Mohamad I. Bahua, Mohamad Lihawa, Nikmah Musa, and Fauzan Zakaria

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.

Submitted:

Published:

ISSN: 2684-1827

DOI:

H. Gubali
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: hayatiningsihgubali@ung.ac.id)

Z. Ilahude
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: zulzainilahude@ung.ac.id)

M. I. Bahua*
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: mohamad.bahua@ung.ac.id)

M. Lihawa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: mohamd.lihawa@ung.ac.id)

N. Musa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: nikmahmusa@ung.ac.id)

F. Zakaria
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: fauzanzakaria@ung.ac.id)

Corresponding author

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, Phospor, 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×b×c×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:

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

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 α=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 *Azospirilum* 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 N_2 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	Soil Sample		
Parameter	P0	P3	P5
pH (H2O)	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).

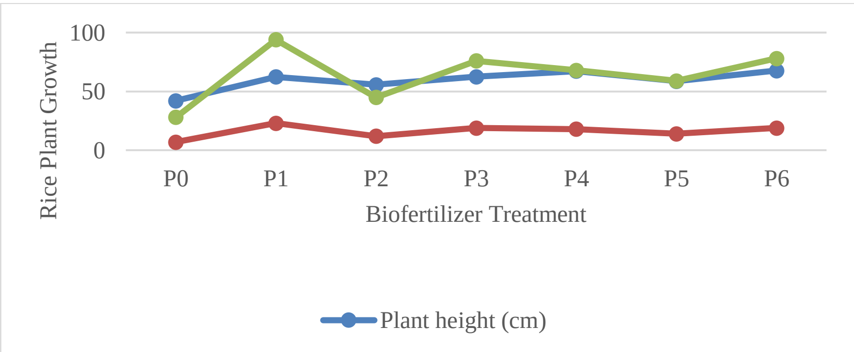


Fig. 1. Components of Rice Plant Growth in All Treatments.

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.

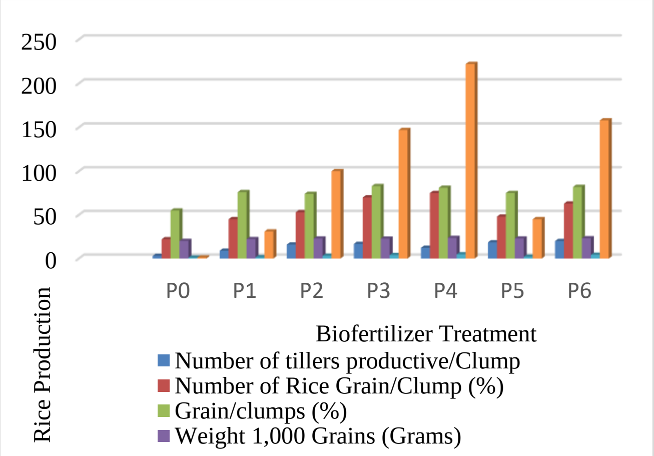


Fig. 2. Components of Rice Production in All Treatments.

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.

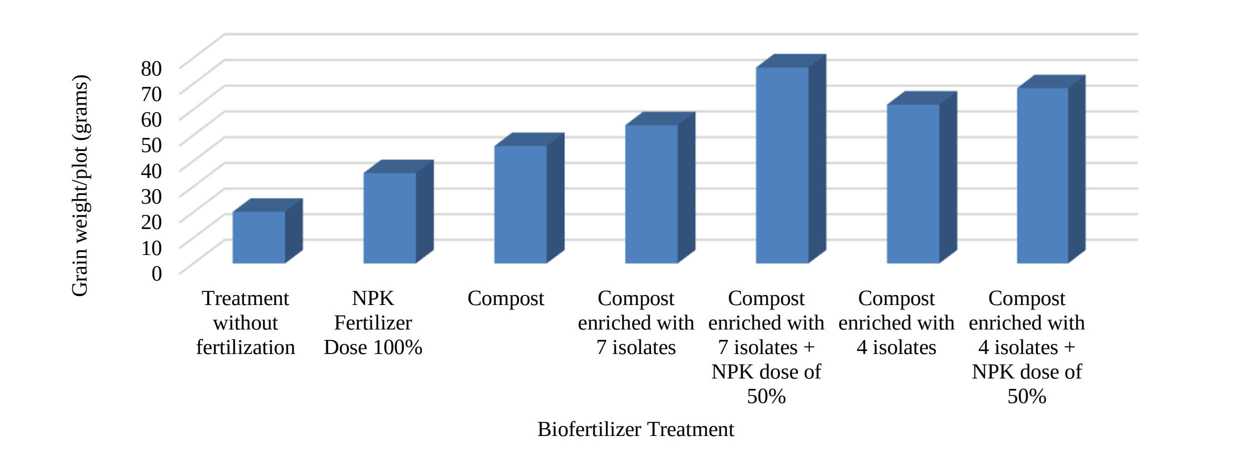


Fig. 3. Production of Dry Unhulled Rice/Plots in All Treatments.

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.

Acknowledgment

The research would like to thank the Government of North Gorontalo District, Gorontalo Province, Indonesia, for allowing us to conduct research on the Agricultural Office Land.

Funding

Thank you to the Head of the Department of Agrotechnology, Faculty of Agriculture, Gorontalo State University for funding this research.

Conflict of Interest

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

References

[1] Sharma, B., Tiwari, S., Kumawat, KC & Cardinale, M. Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations. *Journal Science of The Total Environment*. 2023; 860 (2). 476–488. https://doi.org/10.1016/j.scitotenv.2022.160476.

[2] Sayed, EG & Ouis, MA. Improvement of pea plant growth, yield, and seed quality using glass fertilizers and biofertilizers. *Journal of Environmental Technology & Innovation*. 2022; 26(5). 356–368. https://doi.org/10.1016/j.eti.2022.102356.

[3] Mahmud, K., Missaoui, A., Lee, K., Ghimire, B., Presley, HW & Makaju, S. Rhizosphere microbiome manipulation for sustainable crop production. *Journal of Current Plant Biology*. 2021; 27 (9). 210–225. https://doi.org/10.1016/j.cpb.2021.100210.

[4] Kulsum, PGPS, Khanam, R., Das, S., Nayak, AK, Tack, FMG, Meers, E., Vithanage, M., Shahid, M., Kumar, A., Chakraborty, S., Bhattacharya, T. & Biswas, JK. A state-of-the-art review on cadmium uptake, toxicity, and tolerance in rice: From physiological response to remediation process. *Journal of Environmental Research*. 2023; 220 (3). 115–127. https://doi.org/10.1016/j.envres.2022.115098.

[5] Dos Santos, SRL, Costa, RM, De Aviz, RO, Melo, VMM, Lopes, ACA, Pereira, APA, Mendes, LW, Barbosa, RS & Araujo, ASF. Differential plant growth-promoting rhizobacteria species selection by maize, cowpea, and lima bean. *Journal of the Rhizosphere*. 2022; 24 (12). 626–639. https://doi.org/10.1016/j.rhisph.2022.100626.

[6] Cao, TND, Mukhtar, H., Le, LT, Tran, DPH, Ngo, MTT, Pham, MDT, Nguyen, TB, Quyen Vo, TK & Bui, XT. Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus. *Journal Science of The Total Environment*. 2023; 870 (4). 161–174. https://doi.org/10.1016/j.scitotenv.2023.161927.

[7] El-Sobky, ESEA, Taha, AE, El-Sharnouby, M., Sayed, SM & Elrys, USA. Zinc-biochemical co-fertilization improves rice performance and reduces nutrient surplus under semi-arid environmental conditions. *Saudi Journal of Biological Sciences*. 2022; 29 (3). 1653–1667. https://doi.org/10.1016/j.sjbs.2021.10.066.

[8] Kumar, S., Sindhu, SS & Kumar, R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Journal of Current Research in Microbial Sciences*. 2022; 3 (4). 100–112. https://doi.org/10.1016/j.crmicr.2021.100094.

[9] Zhang, C., Li, Q., Feng, R., Zhang, Z., Yang, Y. & Liu, J. C: N:P stoichiometry of plant-soil-microbes in the secondary succession of zokor-made mounds on the Qinghai-Tibet Plateau. *Journal of Environmental Research*. 2023. 222 (4). 115–127. https://doi.org/10.1016/j.envres.2023.115333.

[10] Arora, S., Murmu, G., Mukherjee, K., Saha, S. & Maity, D. A comprehensive overview of nanotechnology in sustainable agriculture. *Journal of Biotechnology*. 2022; 355 (8). 21–41.https://doi.org/10.1016/j.jbiotec.2022.06.007.

[11] Paravar, A., Piri, R., Balouchi, H. & Ma, Y. Microbial seed coating: An attractive tool for sustainable agriculture. *Journal Biotechnology Reports*. 2023; 37(3). 781–795. https://doi.org/10.1016/j.btre.2023.e00781.

[12] Xiao, J., Wang, G., Liu, H. & Dai, X. Application of composted lipstatin fermentation residue as organic fertilizer: Temporal changes in soil characteristics and bacterial community. *Chemosphere Journal*. 2022; 306 (11). 135–148. https://doi.org/10.1016/j.chemosphere.2022.135637.

[13] Das, PP, Singh, KRB, Nagpure, G., Mansoori, A., Singh, RP, Ghazi, IA, Kumar, A. & Singh, J.. Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Journal of Environmental Research*. 2022; 214 (11). 821–836.https://doi.org/10.1016/j.envres.2022.113821.

[14] Gao. Q., Tao, D., Qi, Z., Liu, Y., Guo, J. & Yu, Y. Amidoxime functionalized PVDF-based chelating membranes enable synchronous elimination of heavy metals and organic contaminants from wastewater. *Journal of Environmental Management*. 2022; 318 (9). 564–578. https://doi.org/10.1016/j.jenvman.2022.115643.

[15] Chen, K., Liang, J., Xu, X., Zhao, L., Qiu, H., Wang, X. & Cao, X. Roles of soil active constituents in the degradation of sulfamethoxazole by biochar/persulfate: Contrasting effects of iron minerals and organic matter. *Journal Science of The Total Environment*. 2022; 853 (12). 532–548. https://doi.org/10.1016/j.scitotenv.2022.158532.

[16] Jafari, F., Khademi, H., Shahrokh, V., Cano, AF, Acosta, JA & Khormali, F. Biological weathering of phlogopite during enriched vermicomposting. *Journal Pedosphere*. 2021; 31 (6). 450–451. https://doi.org/10.1016/S1002-0160(20)60083-2.

[17] Rasines, L., Miguel, GS, Garcia, AM, Hernandez, FA, Hontoria, E. & Aguayo, E. Optimizing the environmental sustainability of alternative post-harvest scenarios for fresh vegetables: A case study in Spain. *Journal Science of The Total Environment.* 2023; 860 (2). 16–30. https://doi.org/10.1016/j.scitotenv.2022.160422.

[18] Ladha, JK, Peoples, MB, Reddy, PM, Biswas, JC, Bennett, A., Jat, ML & Krupnik, TJ. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Journal of Field Crops Research.* 2022; 283 (7). 854–868. https://doi.org/10.1016/j.fcr.2022.108541.

[19] Zhou, L., Xue, J., Xu, Y., Tian, W., Huang, G., Liu, L. & Zhang, Y. The effect of biochar addition on copper and zinc passivation pathways mediated by humification and microbial community evolution during pig manure composting. *Journal of Bioresource Technology.* 2023; 370 (2). 128–138. https://doi.org/10.1016/j.biortech.2023.128575.

[20] Zhao, M., Wu, H., Lu, J., Sun, G. & Du, L. Effect of grain size on mechanical property and corrosion behavior of a metastable austenitic stainless steel. *Journal Materials Characterization.* 2022; 194 (12). 360–372. https://doi.org/10.1016/j.matchar.2022.112360.

[21] Taheri, E., Tarighi, S. & Taheri, P. Characterization of root endophytic Paenibacillus polymyxa isolates with biocontrol activity against Xanthomonas translucens and Fusarium graminearum. *Journal of Biological Control*. 2022; 174 (11). 503–515. https://doi.org/10.1016/j.biocontrol.2022.105031.

[22] Singh, B., Sahu, PM, Aloria, M., Reddy, SS, Prasad, J. & Sharma, RA. Azotobacter chroococcum and Pseudomonas putida enhance pyrroloquinazoline alkaloids accumulation in Adhatoda vasica hairy roots by biotization. *Journal of Biotechnology.* 2022; 353 (7). 51–60. https://doi.org/10.1016/j.jbiotec.2022.05.011.

[23] Emmanuel, OC & Babalola, OO. Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. *Journal of Microbiological Research*. 2020; 239 (10). 656–666. https://doi.org/10.1016/j.micres.2020.126569.

[24] Basílio, F., Dias, T., Santana, MM, Melo, J., Carvalho, L., Correia, P. & Cruz, C. Multiple modes of action are needed to unlock soil phosphorus fractions unavailable for plants: The example of bacteria- and fungi-based biofertilizers. *Journal of Applied Soil Ecology*. 2022; 178 (10). 455–466. https://doi.org/10.1016/j.apsoil.2022.104550.

[25] Mosqueda, MCO, Fadiji, AE, Babalola, OO, Glick, BR & Santoyo, G. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. *Journal of Microbiological Research*. 2022; 263 (10). 127–137. https://doi.org/10.1016/j.micres.2022.127137.

[26] Marzouk, SH, Tindwa, HJ, Amuri, NA & Semoka, JM. An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. *Journal Heliyon*. 2023; 9 (1). 13–25. https://doi.org/10.1016/j.heliyon.2023.e13040.

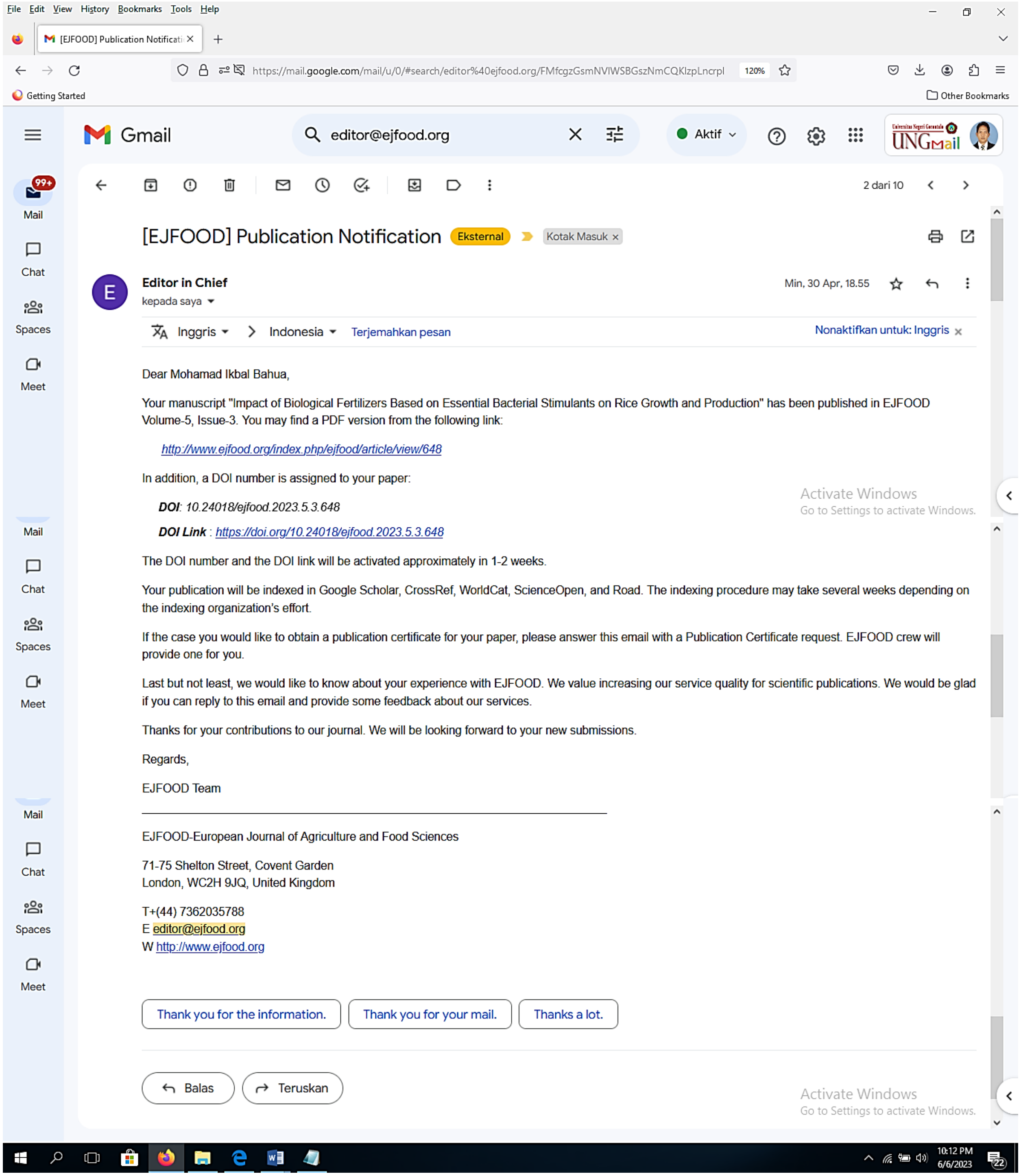
[27] Exposito, CDV, Lopez, JA, Liu, J., Bao, N., Liang, J. & Zhang, J. Development of a cold-active microbial compound biofertilizer on the improvement for rice (Oryza sativa L.) tolerance at low-temperature. *Journal of the Rhizosphere*. 2022; 24 (12). 586–596. https://doi.org/10.1016/j.rhisph.2022.100586.

[28] Prabakaran, S., Mohanraj, T., Arumugam, A. & Sudalai, S. A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Journal of Industrial Crops and Products*. 2022; 183 (9). 942–954. https://doi.org/10.1016/j.indcrop.2022.114942.

[29] Moulick, D., Ghosh, D., Mandal, J., Bhowmick, S., Mondal, D., Choudhury, S., Santra, SC, Vithanage, M. & Biswas, JK. A cumulative assessment of plant growth stages and selenium supplementation on arsenic and micronutrients accumulation in rice grains. *Journal of Cleaner Production*. 2023; 386 (2). 35–47. https://doi.org/10.1016/j.jclepro.2022.135764.

[30] Danso, F., Agyare, WA & Plange, AB. Benefits and costs of cultivating rice using biochar-inorganic fertilizer combinations. *Journal of Agriculture and Food Research*. 2023; 11 (3). 10–27. https://doi.org/10.1016/j.jafr.2022.100491.

5. Bukti Konfirmasi Artikel Published Online Tanggal 30 April 2023


[EJFOOD] Publication Notification

Editor in Chief

kepada saya

Min, 30 Apr, 18.55

Dear Mohamad Ikbal Bahua,

Your manuscript "Impact of Biological Fertilizers Based on Essential Bacterial Stimulants on Rice Growth and Production" has been published in EJFOOD Volume-5, Issue-3. You may find a PDF version from the following link:

http://www.ejfood.org/index.php/ejfood/article/view/648

In addition, a DOI number is assigned to your paper:

DOI: *10.24018/ejfood.2023.5.3.648*

DOI Link : *https://doi.org/10.24018/ejfood.2023.5.3.648*

The DOI number and the DOI link will be activated approximately in 1-2 weeks.

Your publication will be indexed in Google Scholar, CrossRef, WorldCat, ScienceOpen, and Road. The indexing procedure may take several weeks depending on the indexing organization's effort.

If the case you would like to obtain a publication certificate for your paper, please answer this email with a Publication Certificate request. EJFOOD crew will provide one for you.

Last but not least, we would like to know about your experience with EJFOOD. We value increasing our service quality for scientific publications. We would be glad if you can reply to this email and provide some feedback about our services.

Thanks for your contributions to our journal. We will be looking forward to your new submissions.

Regards,

EJFOOD Team

EJFOOD-European Journal of Agriculture and Food Sciences

71-75 Shelton Street, Covent Garden
London, WC2H 9JQ, United Kingdom

T+(44) 7362035788
E editor@ejfood.org
W http://www.ejfood.org

European Journal of Agriculture and Food Sciences
www.ejfood.org

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

Hayatiningsih Gubali, Zulzain Ilahude, Mohamad I. Bahua, Mohamad Lihawa, Nikmah Musa, and Fauzan Zakaria

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.

Submitted: February 9, 2023
Published: April 30, 2023
ISSN: 2684-1827
DOI: 10.24018/ejfood.2023.5.2.648

H. Gubali
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: hayatiningsihgubali@ung.ac.id)
Z. Ilahude
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: zulzainilahude@ung.ac.id)
M. I. Bahua*
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: mohamad.bahua@ung.ac.id)
M. Lihawa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: mohamd.lihawa@ung.ac.id)
N. Musa
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: nikmahmusa@ung.ac.id)
F. Zakaria
Department of Agrotechnology, Faculty of Agriculture, Universitas Negeri Gorontalo, Indonesia
(e-mail: fauzanzakaria@ung.ac.id)

Corresponding author

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, Phospor, 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×b×c×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:

$$\frac{Treatment - Negative\ Control}{Positive\ Control - Negative\ Control} x\ 100 \qquad (1)$$

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 α=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 *Azospirilum* 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 N_2 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	Soil Sample		
Parameter	P0	P3	P5
pH (H2O)	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).

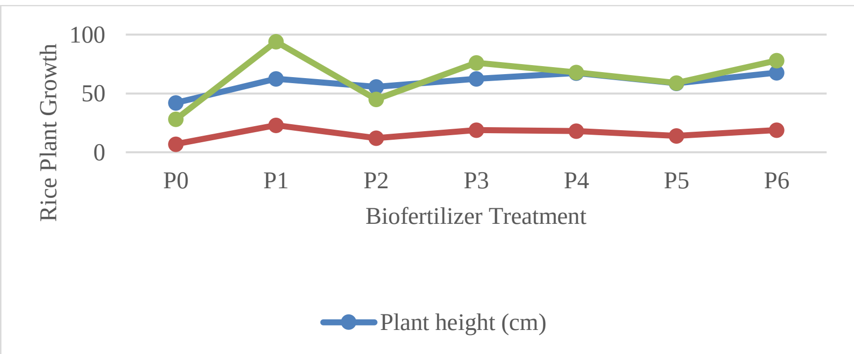


Fig. 1. Components of Rice Plant Growth in All Treatments.

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.

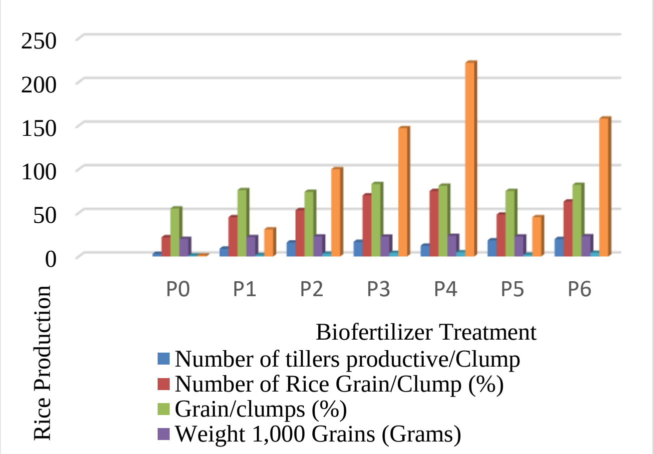


Fig. 2. Components of Rice Production in All Treatments.

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.

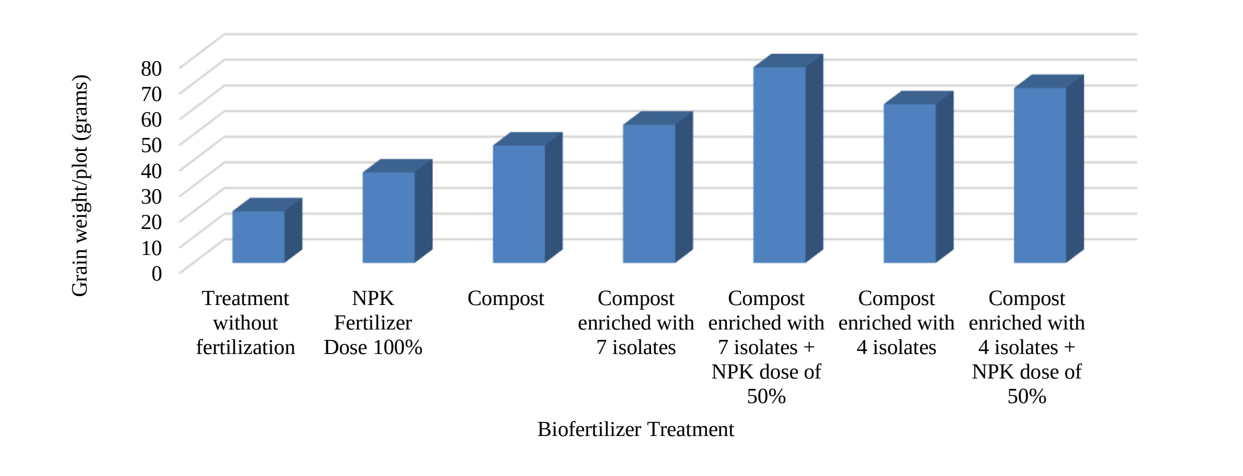


Fig. 3. Production of Dry Unhulled Rice/Plots in All Treatments.

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.

Acknowledgment

The research would like to thank the Government of North Gorontalo District, Gorontalo Province, Indonesia, for allowing us to conduct research on the Agricultural Office Land.

Funding

Thank you to the Head of the Department of Agrotechnology, Faculty of Agriculture, Gorontalo State University for funding this research.

Conflict of Interest

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

References

[1] Sharma, B., Tiwari, S., Kumawat, KC & Cardinale, M. Nano-biofertilizers as bio-emerging strategies for sustainable agriculture development: Potentiality and their limitations. *Journal Science of The Total Environment*. 2023; 860 (2). 476–488. https://doi.org/10.1016/j.scitotenv.2022.160476.

[2] Sayed, EG & Ouis, MA. Improvement of pea plant growth, yield, and seed quality using glass fertilizers and biofertilizers. *Journal of Environmental Technology & Innovation*. 2022; 26(5). 356–368. https://doi.org/10.1016/j.eti.2022.102356.

[3] Mahmud, K., Missaoui, A., Lee, K., Ghimire, B., Presley, HW & Makaju, S. Rhizosphere microbiome manipulation for sustainable crop production. *Journal of Current Plant Biology*. 2021; 27 (9). 210–225. https://doi.org/10.1016/j.cpb.2021.100210.

[4] Kulsum, PGPS, Khanam, R., Das, S., Nayak, AK, Tack, FMG, Meers, E., Vithanage, M., Shahid, M., Kumar, A., Chakraborty, S., Bhattacharya, T. & Biswas, JK. A state-of-the-art review on cadmium uptake, toxicity, and tolerance in rice: From physiological response to remediation process. *Journal of Environmental Research*. 2023; 220 (3). 115–127. https://doi.org/10.1016/j.envres.2022.115098.

[5] Dos Santos, SRL, Costa, RM, De Aviz, RO, Melo, VMM, Lopes, ACA, Pereira, APA, Mendes, LW, Barbosa, RS & Araujo, ASF. Differential plant growth-promoting rhizobacteria species selection by maize, cowpea, and lima bean. *Journal of the Rhizosphere*. 2022; 24 (12). 626–639. https://doi.org/10.1016/j.rhisph.2022.100626.

[6] Cao, TND, Mukhtar, H., Le, LT, Tran, DPH, Ngo, MTT, Pham, MDT, Nguyen, TB, Quyen Vo, TK & Bui, XT. Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus. *Journal Science of The Total Environment*. 2023; 870 (4). 161–174. https://doi.org/10.1016/j.scitotenv.2023.161927.

[7] El-Sobky, ESEA, Taha, AE, El-Sharnouby, M., Sayed, SM & Elrys, USA. Zinc-biochemical co-fertilization improves rice performance and reduces nutrient surplus under semi-arid environmental conditions. *Saudi Journal of Biological Sciences*. 2022; 29 (3). 1653–1667. https://doi.org/10.1016/j.sjbs.2021.10.066.

[8] Kumar, S., Sindhu, SS & Kumar, R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Journal of Current Research in Microbial Sciences*. 2022; 3 (4). 100–112. https://doi.org/10.1016/j.crmicr.2021.100094.

[9] Zhang, C., Li, Q., Feng, R., Zhang, Z., Yang, Y. & Liu, J. C: N:P stoichiometry of plant-soil-microbes in the secondary succession of zokor-made mounds on the Qinghai-Tibet Plateau. *Journal of Environmental Research*. 2023. 222 (4). 115–127. https://doi.org/10.1016/j.envres.2023.115333.

[10] Arora, S., Murmu, G., Mukherjee, K., Saha, S. & Maity, D. A comprehensive overview of nanotechnology in sustainable agriculture. *Journal of Biotechnology*. 2022; 355 (8). 21–41.https://doi.org/10.1016/j.jbiotec.2022.06.007.

[11] Paravar, A., Piri, R., Balouchi, H. & Ma, Y. Microbial seed coating: An attractive tool for sustainable agriculture. *Journal Biotechnology Reports*. 2023; 37(3). 781–795. https://doi.org/10.1016/j.btre.2023.e00781.

[12] Xiao, J., Wang, G., Liu, H. & Dai, X. Application of composted lipstatin fermentation residue as organic fertilizer: Temporal changes in soil characteristics and bacterial community. *Chemosphere Journal*. 2022; 306 (11). 135–148. https://doi.org/10.1016/j.chemosphere.2022.135637.

[13] Das, PP, Singh, KRB, Nagpure, G., Mansoori, A., Singh, RP, Ghazi, IA, Kumar, A. & Singh, J.. Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Journal of Environmental Research*. 2022; 214 (11). 821–836.https://doi.org/10.1016/j.envres.2022.113821.

[14] Gao. Q., Tao, D., Qi, Z., Liu, Y., Guo, J. & Yu, Y. Amidoxime functionalized PVDF-based chelating membranes enable synchronous elimination of heavy metals and organic contaminants from wastewater. *Journal of Environmental Management*. 2022; 318 (9). 564–578. https://doi.org/10.1016/j.jenvman.2022.115643.

[15] Chen, K., Liang, J., Xu, X., Zhao, L., Qiu, H., Wang, X. & Cao, X. Roles of soil active constituents in the degradation of sulfamethoxazole by biochar/persulfate: Contrasting effects of iron minerals and organic matter. *Journal Science of The Total Environment*. 2022; 853 (12). 532–548. https://doi.org/10.1016/j.scitotenv.2022.158532.

[16] Jafari, F., Khademi, H., Shahrokh, V., Cano, AF, Acosta, JA & Khormali, F. Biological weathering of phlogopite during enriched vermicomposting. *Journal Pedosphere*. 2021; 31 (6). 450–451. https://doi.org/10.1016/S1002-0160(20)60083-2.

[17] Rasines, L., Miguel, GS, Garcia, AM, Hernandez, FA, Hontoria, E. & Aguayo, E. Optimizing the environmental sustainability of alternative post-harvest scenarios for fresh vegetables: A case study in Spain. *Journal Science of The Total Environment.* 2023; 860 (2). 16–30. https://doi.org/10.1016/j.scitotenv.2022.160422.

[18] Ladha, JK, Peoples, MB, Reddy, PM, Biswas, JC, Bennett, A., Jat, ML & Krupnik, TJ. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Journal of Field Crops Research.* 2022; 283 (7). 854–868. https://doi.org/10.1016/j.fcr.2022.108541.

[19] Zhou, L., Xue, J., Xu, Y., Tian, W., Huang, G., Liu, L. & Zhang, Y. The effect of biochar addition on copper and zinc passivation pathways mediated by humification and microbial community evolution during pig manure composting. *Journal of Bioresource Technology.* 2023; 370 (2). 128–138. https://doi.org/10.1016/j.biortech.2023.128575.

[20] Zhao, M., Wu, H., Lu, J., Sun, G. & Du, L. Effect of grain size on mechanical property and corrosion behavior of a metastable austenitic stainless steel. *Journal Materials Characterization.* 2022; 194 (12). 360–372. https://doi.org/10.1016/j.matchar.2022.112360.

[21] Taheri, E., Tarighi, S. & Taheri, P. Characterization of root endophytic Paenibacillus polymyxa isolates with biocontrol activity against Xanthomonas translucens and Fusarium graminearum. *Journal of Biological Control*. 2022; 174 (11). 503–515. https://doi.org/10.1016/j.biocontrol.2022.105031.

[22] Singh, B., Sahu, PM, Aloria, M., Reddy, SS, Prasad, J. & Sharma, RA. Azotobacter chroococcum and Pseudomonas putida enhance pyrroloquinazoline alkaloids accumulation in Adhatoda vasica hairy roots by biotization. *Journal of Biotechnology.* 2022; 353 (7). 51–60. https://doi.org/10.1016/j.jbiotec.2022.05.011.

[23] Emmanuel, OC & Babalola, OO. Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. *Journal of Microbiological Research*. 2020; 239 (10). 656–666. https://doi.org/10.1016/j.micres.2020.126569.

[24] Basílio, F., Dias, T., Santana, MM, Melo, J., Carvalho, L., Correia, P. & Cruz, C. Multiple modes of action are needed to unlock soil phosphorus fractions unavailable for plants: The example of bacteria- and fungi-based biofertilizers. *Journal of Applied Soil Ecology*. 2022; 178 (10). 455–466. https://doi.org/10.1016/j.apsoil.2022.104550.

[25] Mosqueda, MCO, Fadiji, AE, Babalola, OO, Glick, BR & Santoyo, G. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. *Journal of Microbiological Research*. 2022; 263 (10). 127–137. https://doi.org/10.1016/j.micres.2022.127137.

[26] Marzouk, SH, Tindwa, HJ, Amuri, NA & Semoka, JM. An overview of underutilized benefits derived from Azolla as a promising biofertilizer in lowland rice production. *Journal Heliyon*. 2023; 9 (1). 13–25. https://doi.org/10.1016/j.heliyon.2023.e13040.

[27] Exposito, CDV, Lopez, JA, Liu, J., Bao, N., Liang, J. & Zhang, J. Development of a cold-active microbial compound biofertilizer on the improvement for rice (Oryza sativa L.) tolerance at low-temperature. *Journal of the Rhizosphere*. 2022; 24 (12). 586–596. https://doi.org/10.1016/j.rhisph.2022.100586.

[28] Prabakaran, S., Mohanraj, T., Arumugam, A. & Sudalai, S. A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Journal of Industrial Crops and Products*. 2022; 183 (9). 942–954. https://doi.org/10.1016/j.indcrop.2022.114942.

[29] Moulick, D., Ghosh, D., Mandal, J., Bhowmick, S., Mondal, D., Choudhury, S., Santra, SC, Vithanage, M. & Biswas, JK. A cumulative assessment of plant growth stages and selenium supplementation on arsenic and micronutrients accumulation in rice grains. *Journal of Cleaner Production*. 2023; 386 (2). 35–47. https://doi.org/10.1016/j.jclepro.2022.135764.

[30] Danso, F., Agyare, WA & Plange, AB. Benefits and costs of cultivating rice using biochar-inorganic fertilizer combinations. *Journal of Agriculture and Food Research*. 2023; 11 (3). 10–27. https://doi.org/10.1016/j.jafr.2022.100491.