



Application of Biofertilizer Combined with Bioactivator in the Vegetative Phase of Inpari 32 Rice Variety

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Abstract. Sustainable rice cultivation requires effective nutrient management strategies to reduce chemical fertilizer dependency. Biofertilizers enriched with humic acid and microbial consortia offer an eco-friendly alternative to enhance soil fertility and plant productivity. This study evaluated the effects of biofertilizer combined with humic acid and a microbial consortium on vegetative growth, chlorophyll content, and macronutrient availability in Inpari 32 rice. The experiment was conducted in a greenhouse at Banyuwangi State Polytechnic using a completely randomized factorial design with two biofertilizer types: biofertilizer + humic acid (B1) and biofertilizer + microbial consortium (*Bacillus*, *Trichoderma*, *Saccharomyces*) (B2), and three application doses: 300 g, 400 g, and 500 g per pot. Although ANOVA results indicated no statistically significant differences among treatments, a consistent positive trend was observed in plant height, tiller number, and chlorophyll content at the 400 g dose. Soil N, P, and K levels tended to decrease, possibly due to enhanced nutrient uptake by plants, suggesting an improved nutrient use efficiency. Overall, the combination of biofertilizer and humic acid at the 400 g dose tended to show higher values compared to other treatments, although these differences were not statistically significant. These findings support the use of multi-species microbial biofertilizers and IoT-based precision fertilization systems to promote sustainable rice production in Indonesia.

Keywords: Biofertilizer; humic acid; microbial consortium; rice growth; nutrient uptake.

Type of the Paper: Regular Article.



1. Introduction

Rice plant (*Oryza sativa* L.) is a strategic commodity for Indonesia's food security. Efforts to increase rice productivity continue to address the growing national food demand driven by population growth [1]. Globally, farmers use around 115 million tons of chemical fertilizers, of which only 35% is absorbed by plants. The remaining 65% becomes residue and a major soil pollutant, reducing soil fertility, degrading soil structure, and decreasing microbial activity [2]. Current agricultural practices that rely heavily on inorganic phosphate fertilizers also cause environmental damage. These fertilizers are non-renewable and negatively impact the food production chain [3]. Therefore, environmentally friendly fertilization innovations, such as the use of biofertilizers combined with bioactivators such as humic acid and soil microorganisms, are important solutions in sustainable agricultural systems.

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Biofertilizer, or biological fertilizer, is a soil conditioner containing live microorganisms that increase nutrient availability and uptake by plants. These microbes function through various biological mechanisms, such as nitrogen fixation, phosphate solubilization, organic matter decomposition, and the production of plant growth-promoting compounds [4]. According to Indriani et al. [5], the addition of humic acid to biofertilizers can improve soil health, especially by increasing carbon storage in soils with low organic carbon content. Humic acid, an active ingredient extracted from organic matter, functions as a growth stimulant. Several microbial genera proven effective as biofertilizers include *Bacillus*, *Trichoderma*, and *Saccharomyces*. Each has different physiological and ecological roles but acts synergistically to enhance rice growth and yield.

Bacillus microbes are a group of rhizosphere bacteria capable of non-symbiotic nitrogen fixation, phosphate solubilization, and the production of phytohormones such as indole-3-acetic acid (IAA) that stimulate root growth [6]. In addition, several species of *Bacillus subtilis* function as biocontrol agents against soil pathogens, thereby suppressing root diseases and strengthening plant resistance [7].

The use of biofertilizers can reduce farmers' dependence on chemical fertilizers, which are known to pollute the environment. *Trichoderma* spp. are among the most widely used and commercially produced fungi for biofertilizers [8]. *Trichoderma* is a saprophytic fungus effective in decomposing organic matter and releasing macro- and micronutrients bound in soil complexes. This fungus also acts as a *plant growth-promoting fungus* (PGPF) through the production of hydrolytic enzymes, siderophores, and plant hormones such as gibberellin and auxin [9]. The application of *Trichoderma* has been proven to increase soil microbial activity, improve soil structure, and enhance rice root growth [10].

While the roles of beneficial microbes such as *Bacillus* and *Trichoderma* in improving soil fertility and plant growth are well documented, the use of *Saccharomyces* in multi-microbial biofertilizer systems remains limited. Moreover, its combined application with humic acid has been insufficiently explored, particularly regarding nutrient use efficiency and rice productivity. Therefore, this study evaluates the combined application of biofertilizer containing *Saccharomyces*, *Bacillus*, and *Trichoderma* with humic acid at different rates. The novelty of this study lies in assessing their potential synergistic effects on plant growth, soil nutrient dynamics, and crop performance [11].

Therefore, research on the application of biofertilizers containing humic acid and beneficial microbes in rice cultivation is needed to evaluate their effectiveness in enhancing growth and yield, as well as improving soil biological conditions. The results of this study is expected to provide a scientific basis for developing multimicrobial biofertilizer technology as an alternative to balanced

chemical fertilization, improving fertilization efficiency, and supporting a sustainable rice farming system in Indonesia. This study aims to evaluate the effectiveness of biofertilizers containing humic acid combined with *Bacillus* sp., *Trichoderma* sp, and *Saccharomyces* on the growth, yield, and soil biological properties of rice (*Oryza sativa* L.) under field conditions in Indonesia. The findings are expected to provide a scientific foundation for the development of multimicrobial biofertilizer technology as a sustainable alternative to chemical fertilizers, thereby improving fertilization efficiency and supporting rice farming systems in Indonesia.

2. Materials and Methods

2.1. Study Site and Duration

Research on the application of biofertilizer to rice plants was conducted in the greenhouse of the Agribusiness Department at Banyuwangi State Polytechnic. Observations of production parameters were conducted in the field and in the Plant Production Laboratory, Agribusiness Study Program, Department of Agriculture, State Polytechnic of Banyuwangi. The research period (data collection) lasted approximately 3 months in the field.

2.2. Experimental Materials and Equipment

The required equipment include scissors, mortar, test tubes, measuring cups, vortex, centrifuge, and spectrophotometer. PUTS (Rice Field Soil Testing Device), digital scale, analytical balance. The materials required are Inpari 32 seeds, goat biofertilizer with humic acid, goat biofertilizer with *Bacillus* + *Trichoderma* + *Saccharomyces* consortium, 95% ethanol, and distilled water.

2.3. Experimental Design and Treatment Application

The experiment used a completely randomized factorial design consisting of two types of biofertilizers: humic acid biofertilizer (B1) and a microbial consortium biofertilizer containing *Bacillus*, *Trichoderma*, and *Saccharomyces* (B2), with three application doses (300 g/pot, 400 g/pot, and 500 g/pot). In addition, a control treatment (without biofertilizer) was included. Each treatment was replicated three times, resulting in a total of 21 experimental units. Each experimental unit consisted of two plants grown per pot, giving a total plant population of 42. The selection of the 300 g/pot dose as a reference for optimal treatment was based on the findings of Pratiwi et al. [12], which reported its effectiveness when using a microbial consortium as a bioactivator.

Notes:

Control = without biofertilizer

BIT1 = humic acid biofertilizer 300 g

BIT2 = humic acid biofertilizer 400 g

B1T3 = humic acid biofertilizer 500 g

B2T1 = Bacillus + Trichoderma + Saccharomyces consortium biofertilizer 300 g

B2T2 = Bacillus + Trichoderma + Saccharomyces consortium biofertilizer 400 g

B2T3 = Bacillus + Trichoderma + Saccharomyces consortium biofertilizer 500 g

2.2 Research Stages

2.2.1 Preparation Of media

The planting medium consisted of topsoil collected from rice fields and placed in experimental pots. Prior to planting, the soil was analyzed for nitrogen (N), phosphorus (P), and potassium (K) content using a Rice Field Soil Testing Device (PUTS) from the Soil Research Center to determine the initial nutrient status before biofertilizer application. The soil was then air-dried, homogenized, and sieved to remove debris before being transferred into plastic pots (30 cm diameter × 25 cm height), filling approximately two-thirds of each pot. Each pot contained approximately 10 kg of soil.

2.2.2 Seed preparation and planting

Inpari 32 rice seeds were pre-germinated by soaking them in water for 24 hours until the coleoptile emerged. The germinated seeds were then sown in a nursery bed. After approximately 14 days, uniform seedlings were transplanted into the experimental pots containing the prepared soil medium. Two seedlings were planted in each pot.

The biofertilizer used in this study was developed in a previous study conducted in 2023 and described by Pratiwi et al. [12]. The biofertilizer was applied at the beginning of rice planting according to the experimental design. It was applied to the soil based on the designated treatment doses for each experimental unit.

2.2.3 Data Collection and Observations

Vegetative parameters included plant height, number of rice seedlings, chlorophyll content, and flowering age. Vegetative height and growth rate were measured from 7 days after biofertilizer application until the reproductive phase. Chlorophyll content was measured during the maximum vegetative phase (around 30-35 days after planting) using an ethanol extraction method.

Chlorophyll content in rice leaves was measured using the 96% ethanol extraction method. Fresh leaf samples were collected from each treatment, weighed at ±0.1 g, and cut into small pieces. The samples were crushed using a mortar and pestle with the addition of 10 mL of 96% ethanol until homogeneous, then placed in a centrifuge tube. The extract was incubated in dark conditions for 24 hours to ensure complete pigments dissolution, then centrifuged at 1000 rpm for 10 minutes. The resulting clear supernatant was used for spectrophotometric analysis. Measurements were taken at wavelengths of 649 nm and 665 nm using a UV-Vis

spectrophotometer, with ethanol used as a blank. The concentrations of chlorophyll a and b were calculated using the Lichtenthaler Eq. (1), (2), (3) as follows:

$$Chl\ a = 13.36 \times A_{665} - 5.19 \times A_{649} \quad (1)$$

$$Chl\ b = 27.43 \times A_{649} - 8.12 \times A_{665}, \quad (2)$$

$$Chl\ total = 20.0 \times A_{649} + 6.10 \times A_{665} \quad (3)$$

Each treatment was performed with three replicates, and the results were statistically analyzed using analysis of variance (ANOVA) to determine the effect of biofertilizer application on the chlorophyll content of rice leaves.

Plant height was measured non-destructively using a laser distance meter to obtain more accurate and efficient results. Observations were conducted periodically at each vegetative growth stage, starting from 2 weeks after planting until just before flowering, at two-week intervals. Sample plants were randomly selected from each treatment plot, with three to five plants per replicate representing the population. Measurements were taken by placing the laser meter perpendicular to the plant, from the soil surface to tip of the highest leaf actively photosynthesizing leaf. Each measurement was conducted at the same time each day, between 07:00 and 09:00 a.m., to minimize the effects of temperature, light intensity, and plant turgor conditions variations. The plant height values from each replicate were recorded and averaged for each treatment.

The number of rice seedlings was observed to assess the vegetative growth response of rice plants to biofertilizer application. This parameter was observed non-destructively on randomly selected sample plants from each treatment plot. Observations were conducted at 28 days after planting, when the plants were entering the early generative phase. The number of rice seedlings was counted manually by counting all tillers.

Measurement of N, P, and K nutrient levels using IoT-based sensors was performed based on electrochemical and optical ion-detection principles integrated with a microcontroller (ESP32). Ion-selective electrode (ISE) sensors were used to detect nitrate (NO_3^-) and potassium (K^+) ions, while an optical sensor was used for phosphate (PO_4^{3-}) detection.

Soil samples were first extracted using chemical extractants specific to each nutrient, and the resulting extract solutions were measured using the calibrated sensors. Sensor calibration was conducted using standard solutions with known concentrations of N, P, and K to generate calibration curves prior to measurement.

To ensure accuracy, sensor measurements were validated by comparing the results with standard laboratory methods, including spectrophotometry for phosphorus and conventional soil analysis methods for nitrogen and potassium. Sensor output signals were converted into nutrient

concentrations expressed in ppm (parts per million), and all measurement data were recorded digitally through the IoT system.

The data were analyzed using ANOVA. If significant differences were found, further analysis was conducted using the 5% Tukey's Honestly Significant Difference (HSD) test with SPSS Statistic 31 version.

3. Results and Discussion

Observations during the vegetative phase of rice plants included plant height at 14, 21, and 28 days after planting (DAP), as well as the Number of rice seedlings at 28 DAP. Physiological observations in this study included chlorophyll content at the end of the vegetative phase (30 DAP), while soil nutrient content was also analyzed at 30 DAP.

3.1 Plant Height

Plant height is an important parameter in assessing the vegetative growth of rice plants. Environmental factors and nutrient availability greatly affect plant height, especially in the early stages of growth. The application of biofertilizers or biological fertilizers can increase plant height by improving soil fertility and increasing the availability of macro nutrients such as nitrogen, phosphorus, and potassium. Microorganisms in biofertilizers actively contribute to nitrogen fixation, dissolving phosphate, and producing growth hormones such as auxin and cytokinin, which stimulate stem elongation. Thus, the application of biofertilizers is expected to increase rice plant growth and productivity in a sustainable manner [13].

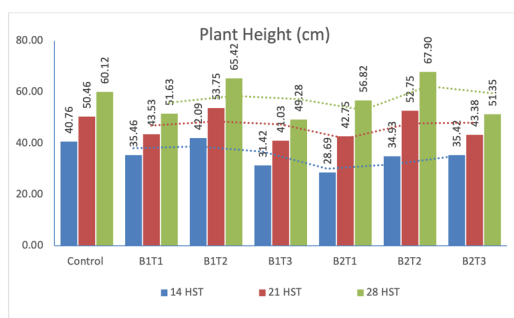


Fig. 1. Plant Height of Plants due to Treatment of Biofertilizer Combined with Bioactivator at 14 – 28 DAP

As shown in Fig. 1, plant height increased progressively at 14, 21, and 28 days after planting (DAP), with significant differences observed between the humic acid-based fertilizer treatment (B1) and the bacterial consortium-based fertilizer treatment (B2). The control treatment had the lowest growth, with an average plant height of only 60.12 cm at 28 d. Conversely, treatments B1T2 and B2T2 produced the highest growth, reaching 65.42 cm and 67.90 cm, respectively, at 28 HSP.

This indicates that the application of solid organic fertilizer, whether containing humic acid or bacteria, plays an important role in enhancing the vegetative growth of rice plants.

The increase in plant height in treatment B1 (humic acid) was attributed to the ability of humic acid to improve the physical, chemical, and biological properties of the soil. Humic acid functions as an active colloid that increases cation exchange capacity (CEC), improves soil structure, and increases the availability of macro nutrients such as nitrogen (N), phosphorus (P), and potassium (K). According to Bertham et al. [14], the application of humic acid can increase N uptake by 20–25% due to its ability to form complexes with metal ions, thereby improving nutrient availability for plant absorption. In addition, humic acid can stimulate enzyme activity and increase root respiration, ultimately promoting the growth of rice plant stems and leaves.

Meanwhile, treatment B2 (solid organic fertilizer with bacteria) had a positive effect due to the presence of microorganisms such as *Azotobacter sp.*, *Bacillus sp.*, and *Pseudomonas sp.*, which are capable of fixing nitrogen from the air, dissolving unavailable phosphates, and producing growth regulators (phytohormones) such as auxin and cytokinin. This microbial activity helps increase nutrient availability and stimulate plant tissue growth. According to Naher et al. [15], the use of microbe-based organic fertilizer can increase rice plant height by up to 30% compared to the control, due to increased nutrient availability and better soil biological activity.

Among the three doses administered (T1 = 300 g, T2 = 400 g, and T3 = 500 g), the 400 g dose (T2) produced the highest plant growth in both treatments B1 and B2. This indicates that 400g is the optimal amount for providing nutrients without excess, which may inhibit nutrient absorption. A lower dose (T1) was unable to meet the nutrient requirements, while a higher dose (T3) may cause ion imbalance in the soil and reduce root absorption efficiency. Therefore, the medium dose (T2) provides the best balance between nutrient availability and plant requirements.

In general, the increase in rice plant height under the humic acid and bacterial fertilizer treatments indicates that both materials play a synergistic role in improving soil fertility and stimulating vegetative growth. Humic acid improves soil structure and nutrient absorption efficiency, while microbes activate biological processes that increase nutrient availability. The combination of these factors results in faster stem growth, longer leaves, and more optimal plant height.

The results of this study support the findings of Wang et al. [16], who reported that the application of humic acid-based organic fertilizers and microbes can increase rice growth through increased soil enzyme activity and the absorption of N and P nutrients. Thus, the application of humic acid-based solid organic fertilizers and bacteria at optimal doses can be a sustainable strategy to increase rice growth and productivity without excessive dependence on inorganic fertilizers.

3.2 Number of rice tillers

The number of rice seedlings is an important indicator of rice yield potential because each productive tiller can form panicles and grains. Tillering is greatly influenced by nutrient availability, plant physiological activity, and soil conditions. The application of biofertilizers plays an important role in increasing the number of rice seedlings by increasing the availability of macro nutrients (N, P, K) and improving soil biological properties. Microorganisms in biofertilizers, such as *Azospirillum*, *Bacillus*, and *Pseudomonas*, are capable of fixing atmospheric nitrogen, dissolving phosphate, and producing phytohormones such as auxin and cytokinin, which stimulate the formation of lateral shoots in rice plants.

Biofertilizers increase nutrient availability and enhance hormonal balance in plants, thereby contributing to tiller initiation. Furthermore, Basu et al. [17] reported that biofertilizer application significantly increased the number of productive rice tillers by improving nitrogen absorption efficiency and promoting soil microbial activity. Similarly, Wei et al. [18] demonstrated that consortium biofertilizer increased the number of rice seedlings and crop yield by up to 25% compared with the control.

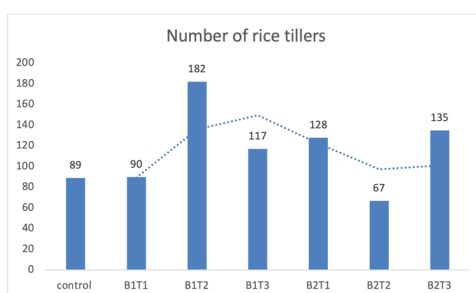


Fig. 2. Graph Number of Rice tillers of Plants due to Treatment of Biofertilizer Combined with Bioactivator at 30 DAP

Fig. 2 illustrates a significant difference in the number of rice seedlings between the biofertilizer treatments and the control. Rice plants in the control group, which did not receive biofertilizer or humic acid, produced an average of 4.00 tillers. In contrast, treatments with biofertilizer plus humic acid (B1) and biofertilizer plus bacterial consortium (B2) resulted in a marked increase in the number of rice seedlings. The highest number of tillers was observed in B2T2 (biofertilizer plus 400 g bacterial consortium), with 6.00 tillers, followed by B1T2 with 5.00 tillers. Treatments with 300 g (T1) and 500 g (T3) doses yielded lower results, as shown in Fig. 2. The increase in tillers compared to the control was attributed to higher availability of macro nutrients, especially nitrogen (N) and phosphorus (P), which are essential for shoot growth and tiller formation. In the control group, plant growth was limited by low soil nutrient availability, resulting in suboptimal tiller formation. This aligns with the findings of Anisuzzaman et al. [19],

who reported that rice plants require sufficient nitrogen availability to support productive tiller formation.

Treatment B1 (biofertilizer + humic acid) showed an increase in the number of rice seedlings compared to the control because humic acid can improve soil structure, increase cation exchange capacity (CEC), and stimulate soil microbial activity. Humic acid also increases nutrient uptake and activates growth hormones that contribute to lateral shoot formation. According to Rahayu et al. [20], the application of humic acid can increase the number of rice tillers by up to 20% through increased root physiological activity and nutrient uptake efficiency.

Meanwhile, treatment B2 (biofertilizer + bacterial consortium) showed the greatest improvement compared to the control. Microorganisms such as *Azotobacter sp.*, *Bacillus sp.*, and *Pseudomonas sp.* in biofertilizers play a role in fixing atmospheric nitrogen, dissolving phosphate, and producing growth regulators such as auxin, cytokinin, and gibberellin, which directly stimulate tiller formation. These results align with the findings of Fitriani et al. [21], who reported that microbe-based biofertilizers can increase the number of rice seedlings and rice productivity through improved soil biological fertility.

A dose of 400 g (T2) proved to be the most optimal compared to 300 g (T1) and 500 g (T3). Low doses were unable to provide sufficient nutrients and microbial activity, while high doses may reduce effectiveness due to potential nutrient imbalances and microbial competition in the root zone. This finding is reinforced by Sakpirom et al. [22], who stated that the application of biofertilizer at a moderate dose can increase the number of rice seedlings by up to 40% compared to the control without causing nutritional stress.

Overall, these results show that the combination of biofertilizer, especially at a 400 g dose of bacterial consortium, is significantly more effective than the control in increasing the number of rice seedlings. This proves that biofertilizer can be a sustainable alternative to replace inorganic fertilizers in modern rice cultivation systems.

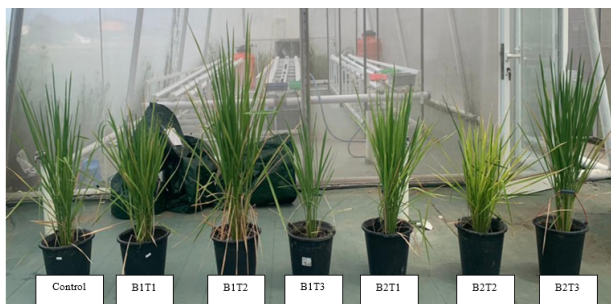


Fig. 3. Number of Rice seedlings of Plants due to Treatment of Biofertilizer Combined with Bioactivator at 30 DAP

Fig. 3 presents a visual comparison of rice seedling growth at 30 days after planting was conducted across seven treatments: the control, B1T1-B1T3 (biofertilizer with humic acid at 300g, 400g, and 500g), and B2T1-B2T3 (biofertilizer with bacterial consortium at corresponding doses). Distinct differences are evident in plant height, tiller number, and leaf color intensity. The B2T2 treatment demonstrates the most pronounced tillering and darker green foliage, indicating enhanced nitrogen uptake and chlorophyll development. Seedlings treated with biofertilizer combinations exhibit more vigorous growth and a denser canopy than the control. These findings indicate that beneficial microorganisms improve nutrient availability and hormonal regulation during the critical vegetative growth phase. The photographic evidence qualitatively corroborates the positive effects of biofertilizer application on rice seedling establishment and tillering capacity, both of which are essential determinants of final grain yield potential.

3.3 Chlorophyll Content

Chlorophyll content testing is used to assess photosynthetic efficiency and plant physiological conditions. Chlorophyll absorbs light energy used in the biomass formation process, making chlorophyll levels an important indicator of plant growth. Biofertilizer application can increase chlorophyll content by improving nitrogen and magnesium availability and stimulating growth hormone production through nitrogen-fixing and phosphate-solubilizing microbes. In addition to improving soil fertility, biofertilizers also increase photosynthetic efficiency and increase plant resistance to environmental stress [23]. Thus, biofertilizers are important in sustainable agriculture and increasing rice productivity.

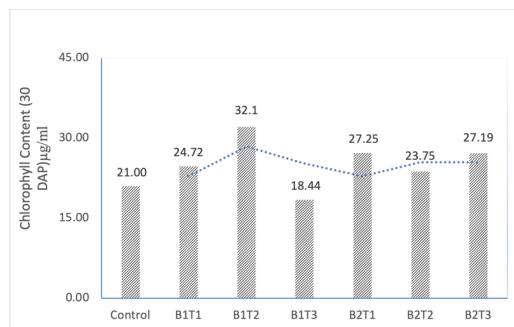


Fig. 4. Graph Chlorophyll Content of Plants Phase Vegetative

Fig. 4 shows that both biofertilizer treatments, combined with humic acid (B1) and a bacterial consortium (B2), resulted in variations in rice leaf chlorophyll content at 30 days after planting (DAP). Chlorophyll content ranged from 18.44 µg/ml to 32.10 µg/ml, while the control group measured 21.00 µg/ml. The B1T2 treatment (biofertilizer plus 400 g humic acid) exhibited the highest chlorophyll content at 32.10 µg/ml, whereas B1T3 (500 g) recorded the lowest at 18.44 µg/ml. However, analysis of variance (ANOVA) and the data in Fig. 4 indicate that these

differences were not statistically significant, suggesting that the observed variations were within the range of natural fluctuations and did not demonstrate a substantial effect on chlorophyll content.

The insignificance of these results may result from several factors. First, biofertilizers require time to adapt to the soil environment to stabilize the microbial population and initiate effective nutrient uptake processes. Nitrogen-fixing microbes such as *Azospirillum* and *Azotobacter* require certain conditions of humidity and organic carbon availability to achieve optimal biological activity. Second, chlorophyll content is greatly influenced by environmental factors such as light intensity, humidity, and nitrogen and magnesium nutrient conditions in the soil. When these conditions are suboptimal or vary plots, the effect of biofertilizer treatment on chlorophyll content may be masked by environmental variability.

Although statistically insignificant, the upward trend in chlorophyll content in treatments B1T2 and B2T2 indicates the potential positive effect of biofertilizers on plant photosynthetic efficiency. Bayat et al. [24] reported that humic acid can increase chlorophyll content by improving the absorption of micronutrients such as Fe and Mg and by increasing leaf enzymatic activity. Meanwhile, Kong and Liu [25] explained that bacterial consortia containing *Bacillus* and *Pseudomonas* produce growth regulators such as cytokinin and auxin, which can slow down chlorophyll degradation during vegetative growth.

In addition, the lack of statistical significance may also be influenced by suboptimal dosage levels. A dose of 300 g may be insufficient to produce a noticeable physiological effect, while a dose of 500 g may cause microbial imbalance and decreased nutrient efficiency due to competition among microbial species. He et al. [26] found that the effectiveness of biofertilizer on chlorophyll content becomes significant only at an optimal dose that was balanced with the biological capacity of the soil and plant nutrient requirements. This is illustrated in Fig. 5.

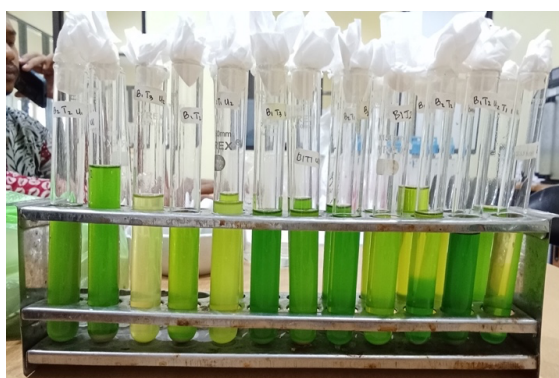


Fig. 5. Chlorophyll Extracts Showing Different Color Intensities Under Various Treatments

Physiologically, the relatively stable chlorophyll content between treatments indicates that all treatments were able to maintain photosynthesis at normal levels, with no significant difference compared with the control. This indicates that the plants remained healthy and that the biofertilizer functioned as a supplementary input rather than a primary source of nitrogen for the leaves in the early stages of growth.

Thus, although the ANOVA results showed no significant differences, the data trends indicate that the combination of biofertilizer with humic acid or bacterial consortium at a moderate dose (400 g) has the potential to increase photosynthetic efficiency. Further research with longer application durations, more controlled environmental conditions, and a greater number of repetitions is needed to verify the significant effect of biofertilizer on the chlorophyll content of rice plants.

3.4 Nutrient Content

The nutrient content of soil media is a major factor determining plant growth and productivity. Macroelements such as nitrogen (N), phosphorus (P), and potassium (K) play an important role in protein formation, metabolic energy, and plant osmotic regulation. Increased nutrient availability can be influenced by soil microorganism activity, organic matter, and the use of biofertilizers that improve the nutrient cycle through biological fixation and phosphate solubilization. According to Walia et al. [27], the sustainable application of biofertilizers can increase the N, P, and K content of the soil and improve long-term fertility.

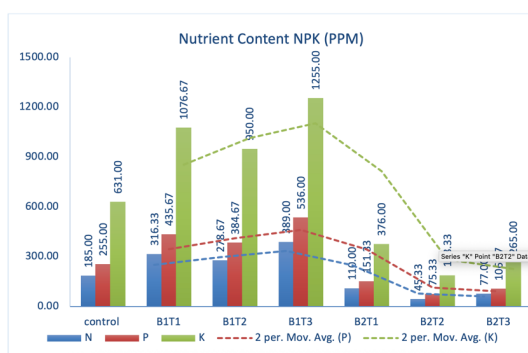


Fig. 6. Nutrient Content in Growing Media (ppm)

Fig. 6 demonstrates that this relative increase is closely associated with the roles of biofertilizers and humic acid in enhancing soil microbial activity and nutrient availability. According to Wang et al. [16], biofertilizers promote biological nitrogen fixation, facilitate phosphate solubilization, and enhance potassium mobility through the activity of microorganisms such as *Azospirillum*, *Bacillus*, and *Pseudomonas*. Additionally, humic acid functions as a chelating agent, thereby increasing nutrient absorption by plant roots.

The relatively lower nutrient content in the soil medium in several treatments, particularly in B2T3 (Fig. 6), may result from active nutrient uptake by plants. This suggests that although soil concentrations of nitrogen, phosphorus, and potassium decreased, these nutrients were efficiently utilized to support vegetative growth. Enhanced nutrient uptake by plants is typically accompanied by reduced soil nutrient concentrations, reflecting high nutrient uptake efficiency.

This observation aligns with the findings of Santoyo et al. [28], who reported that biofertilizer-associated microbes increase the availability of soluble nutrients, thereby facilitating more rapid plant absorption. This phenomenon is also supported by good plant performance, such as increased plant height, number of rice seedlings, and chlorophyll content in the biofertilizer treatment. These conditions indicate that although soil nutrient content has decreased, the nutrients were utilized efficiently to support plant growth [29].

Overall, this trend shows that the use of biofertilizers and humic acid enriches the soil medium and increases nutrient absorption efficiency, resulting in improved physiological and morphological performance of plants. This aligns with the report by Salimani et al. [30], which states that biofertilizer can improve nutrient use efficiency and sustain soil fertility, even when the initial statistical effects appear insignificant.

4. Conclusions

The results of this study indicate that the combination of biofertilizer with humic acid and a microbial consortium can improve soil fertility and support the vegetative growth of rice plants, although statistical analysis shows no significant differences. Increased efficiency of N, P, and K nutrient uptake and improved plant morphophysiological performance, confirms that biofertilizers play an important role in strengthening sustainable agricultural systems. The use of IoT sensors for real-time nutrient monitoring represents an advancement in precision agricultural technology. This study recommends an optimal biofertilizer dose of 400 g pot⁻¹ as the most effective formulation. In the future, the development of commercial multi-species microbial biofertilizers and the integration of digital agronomy systems are expected to accelerate the transformation towards environmentally friendly and resource-efficient smart agriculture.

Abbreviations

ANOVA	Analysis of Variance
CEC	Cation Exchange Capacity
DAP	Days After Planting
RFST	Rice Field Soil Testing

Data availability statement

Data will be shared upon request by the readers.

CRedit authorship contribution statement

Mohammad Ali Mudhor: Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Writing - Original Draft, Funding acquisition, Writing - Review & Editing. **Ari Istanti:** Validation, Formal analysis, Data Curation, Methodology, Validation. **Mokhtar Effendi:** Software, Investigation, Data Curation, Visualization. **Vad'aq Babgei Nur Muhammad:** Investigation, Resources. **As'ad Surya Akbar:** Investigation, Resources. **Sukma Septia Ayu:** Investigation, Resources. **Shabrina Amalia Islami:** Investigation, Resources.

Declaration of Competing Interest

The authors declare no competing interests.

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References

- [1] Elita N, Erlinda R, Sari DA, Illahi AK. Peningkatan Kualitas Pupuk Hayati Diperkaya dengan Bakteri Pelarut Kalium, Fosfor dan Penambat Nitrogen. *Agroteknika* 2024;7:655–71. <https://doi.org/10.55043/agroteknika.v7i4.418>
- [2] Maitra D, Roy B, Dan R, Sarkar S, Choudhury S shyam, Mitra arup kumar. A Study on the Application and Efficiency of Novel Biofertilizer On Paddy : A Small-Scale Study 2024;62. https://www.researchgate.net/publication/381614325_A_Study_on_the_Application_and_Efficiency_of_Novel_Biofertilizer_On_Paddy_A_Small-Scale_Study
- [3] Ngui ME, Melati M. Effects of Bacillus Species Biofertilizer Alone and in Combination with Chemical Fertilizers on the Growth and Development of Rice (*Oryza sativa* L.). *Journal of Agriculture and Environmental Sciences* 2023;12. <https://doi.org/10.15640/jaes.v12n2a2>.
- [4] Dhayalan V, Karuppasamy S. Plant growth promoting rhizobacteria in promoting sustainable agriculture. *Global Journal of Environmental Science and Management* 2021;7. <https://doi.org/10.22034/GJESM.2021.03.06>.
- [5] Indriani T, Bertham YH, Hasanudin H, Prasetyo P, Simanihuruk BW. Effect of Humic Acid on Nutrient Availability and Yield of Upland Rice Inoculated with Biofertilizers in the Coastal Area. *TERRA: Journal of Land Restoration* 2022;5. <https://doi.org/10.31186/terra.5.1.15-20>.
- [6] Zaid DS, Li W, Yang S, Li Y, Zaid DS, Li W, et al. Promoter and Biocontrol Against Post-Harvested Fungi 2023. <https://doi.org/10.1128/spectrum.00519-23>
- [7] Sarkodee-Addo E, Tokiwa C, Bonney P, Aboagye DA, Yeboah A, Abebrese SO, et al. Biofertilizer activity of azospirillum sp. B510 on the rice productivity in Ghana. *Microorganisms* 2021;9. <https://doi.org/10.3390/microorganisms9092000>.

- [8] Anhar A, Fifendy M, Hilda Putri D, Amimi S, Aldo W, Ruchi W. Assessing the Effect of *Trichoderma* Spp. on the Growth of Wetland and Dryland Rice Cultivars At Vegetative Stage. *Pak J Bot* 2024;56. [https://doi.org/10.30848/PJB2024-5\(24\)](https://doi.org/10.30848/PJB2024-5(24)).
- [9] Tian Q, Gong Y, Liu S, Ji M, Tang R, Kong D, et al. Endophytic bacterial communities in wild rice (*Oryza officinalis*) and their plant growth-promoting effects on perennial rice. *Front Plant Sci* 2023;14. <https://doi.org/10.3389/fpls.2023.1184489>.
- [10] Sultana A, Hossain SkMdM, Hasan MdM, Howlader NC, Paul S, Yesmin MostS, et al. Bio-control management of foot and root rot disease of lentil as impacted by different formulations of *Trichoderma* product. *Journal of Oasis Agriculture and Sustainable Development* 2025;7. <https://doi.org/10.56027/joasd.102025>.
- [11] Izquierdo J, Arriagada O, Garcia-Pintos G, Ortiz R, Garcia-Pintos M, Garcia-Pintos M. On-farm foliar application of a humic biostimulant increases the yield of rice. *Agron J* 2024;116. <https://doi.org/10.1002/agj2.21641>.
- [12] Pratiwi GR, Nurrahma AHI, Pratiwi E, Yuniarti E, Ikhwan I. The Effect of Biofertilizer on Growth and Yield of Lowland Rice at Alluvial Soil. *Agrosains : Jurnal Penelitian Agronomi* 2024;25. <https://doi.org/10.20961/agsjpa.v25i2.73000>.
https://www.researchgate.net/publication/387676396_The_Effect_of_Biofertilizer_on_Growth_and_Yield_of_Lowland_Rice_at_Alluvial_Soil
- [13] Hussein SR, Abbas SH, Musa AJ. Effect of Biological, Organic, and Mineral Fertilizers on the Growth and Yield Traits of Rice (*Oryza Sativa* L.). *SABRAO J Breed Genet* 2024;56. <https://doi.org/10.54910/sabrao2024.56.6.28>.
- [14] Bertham RRYH, Ningrum EE, Adiprasetyo RT. Pengaruh Pupuk Mikro Majemuk Dan Asam Humat Terhadap Ketersediaan P Dan Hasil Padi Gogo Di Lahan Pesisir. *Jurnal Ilmu-Illmu Pertanian Indonesia* 2022;24. <https://doi.org/10.31186/jipi.24.2.75-81>.
- [15] Naher UA, Biswas JC, Maniruzzaman M, Khan FH, Sarkar MIU, Jahan A, et al. Bio-Organic Fertilizer: A Green Technology to Reduce Synthetic N and P Fertilizer for Rice Production. *Front Plant Sci* 2021;12. <https://doi.org/10.3389/fpls.2021.602052>.
- [16] Wang Y, Peng S, Hua Q, Qiu C, Wu P, Liu X, et al. The Long-Term Effects of Using Phosphate-Solubilizing Bacteria and Photosynthetic Bacteria as Biofertilizers on Peanut Yield and Soil Bacteria Community. *Front Microbiol* 2021;12. <https://doi.org/10.3389/fmicb.2021.693535>.
- [17] Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, et al. Plant growth promoting rhizobacteria (Pgpr) as green bioinoculants: Recent developments, constraints, and prospects. *Sustainability (Switzerland)* 2021;13. <https://doi.org/10.3390/su13031140>.
- [18] Wei X, Xie B, Wan C, Song R, Zhong W, Xin S, et al. Enhancing Soil Health and Plant Growth through Microbial Fertilizers: Mechanisms, Benefits, and Sustainable Agricultural Practices. *Agronomy* 2024;14:e. <https://doi.org/10.3390/agronomy14030609>.
- [19] Anisuzzaman M, Rafii MY, Jaafar NM, Ramlee SI, Ikkal MF, Haque MA. Effect of organic and inorganic fertilizer on the growth and yield components of traditional and improved rice (*Oryza sativa* l.) genotypes in malaysia. *Agronomy* 2021;11. <https://doi.org/10.3390/agronomy11091830>.
- [20] Rahayu RD, Mindari W, Moch. AMA. Pengaruh Kombinasi Silika dan Asam Humat terhadap Ketersediaan Nitrogen dan Pertumbuhan Tanaman Padi pada Tanah Berpasir. *Soilrens* 2022;19. <https://doi.org/10.24198/soilrens.v19i2.38361>.
- [21] Fitriatin BN, Febriani S, Yuniarti A. Application of biofertilizers to increase upland rice growth, soil nitrogen and fertilizer use efficiency. *IOP Conf Ser Earth Environ Sci* 2021;648. <https://doi.org/10.1088/1755-1315/648/1/012138>.
- [22] Sakpirom J, Nunkaew T, Khan E, Kantachote D. Optimization of carriers and packaging for effective biofertilizers to enhance *Oryza sativa* L. growth in paddy soil. *Rhizosphere* 2021;19. <https://doi.org/10.1016/j.rhisph.2021.100383>.

- [23] Mthiyane P, Aycan M, Mitsui T. Integrating Biofertilizers with Organic Fertilizers Enhances Photosynthetic Efficiency and Upregulates Chlorophyll-Related Gene Expression in Rice. *Sustainability (Switzerland)* 2024;16. <https://doi.org/10.3390/su16219297>.
- [24] Bayat H, Shafie F, Aminifard MH, Daghighi S. Comparative effects of humic and fulvic acids as biostimulants on growth, antioxidant activity and nutrient content of yarrow (*Achillea millefolium* L.). *Sci Hort* 2021;279. <https://doi.org/10.1016/j.scienta.2021.109912>.
- [25] Kong Z, Liu H. Modification of Rhizosphere Microbial Communities: A Possible Mechanism of Plant Growth Promoting Rhizobacteria Enhancing Plant Growth and Fitness. *Front Plant Sci* 2022;13. <https://doi.org/10.3389/fpls.2022.920813>.
- [26] He X, Zhu H, Shi A, Wang X. Optimizing Nitrogen Fertilizer Management Enhances Rice Yield, Dry Matter, and Nitrogen Use Efficiency. *Agronomy* 2024;14. <https://doi.org/10.3390/agronomy14050919>.
- [27] Walia SS, Dhaliwal SS, Gill RS, Kaur T, Kaur K, Randhawa MK, et al. Improvement of soil health and nutrient transformations under balanced fertilization with integrated nutrient management in a rice-wheat system in Indo-Gangetic Plains – A 34-year Research outcomes. *Heliyon* 2024;10. <https://doi.org/10.1016/j.heliyon.2024.e25113>.
- [28] Santoyo G, Guzmán-Guzmán P, Parra-Cota FI, De LSS, Orozco-Mosqueda MDC, Glick BR. Plant growth stimulation by microbial consortia. *Agronomy* 2021;11. <https://doi.org/10.3390/agronomy11020219>.
- [29] Dohare KS, Lahagu MP, Waruwu PNK. Peran Mikroorganisme Tanah dalam Meningkatkan Kesehatan Tanah dan Hasil Pertanian Organik. *Hidroponik : Jurnal Ilmu Pertanian Dan Teknologi Dalam Ilmu Tanaman* 2025;2. <https://doi.org/10.62951/hidroponik.v2i1.253>.
- [30] Salimani S, Verma R, Kumar KB, Mehta S. Impact of Biofertilizers and Integrated Nutrient Management on Transplanted Rice for Improving the Growth, Yield and Soil Quality: A Review. *Int J Plant Soil Sci* 2025;37:271–81. <https://doi.org/10.9734/ijpss/2025/v37i55451>.