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RECEIVED 24 April 2026

REVISED 28 May 2026

ACCEPTED 31 May 2026

PUBLISHED 17 June 2026

CITATION

Zakirova S, Davronov K, Barotov A,
Bulanbayeva P, Alibekova A,
Tashbaltaeva S, Matkarimova A,
Allaeva G, Tadjiev A, Urumbayeva G,
Atabayeva M, Asatullayev F, Ismailov R,
Ernazarov Z, Juraeva K, Po'latov L,
Murodov K and Sindarov O (2026)
Microbial nutrient management
approaches for sustainable rice (*Oryza
sativa* L.) productivity and soil health
under field conditions.
Front. Sustain. Food Syst. 10:1864133.
doi: 10.3389/fsufs.2026.1864133

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Ismailov, Ernazarov, Juraeva, Po'latov,
Murodov and Sindarov. This is an
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Microbial nutrient management approaches for sustainable rice (*Oryza sativa* L.) productivity and soil health under field conditions

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Green technologies exert a significant impact on agricultural systems, providing a promising pathway to enhance sustainable crop production, yield, and soil microbial activity, thereby strengthening food security. A two-year field study was conducted to evaluate the effects of two biofertilizers, *Zamin M* and *Simbioz EM-1*, on rice quality parameters and soil fertility in typical serozem soils of Uzbekistan. The experiment comprised four treatments, including the sole application of each biofertilizer and their combined application which were tested against the control. Among the applied treatments, the combined application produced the most pronounced effects, significantly enhancing germination (30.2%), plant height (23.1%), biomass accumulation (46.2%), and harvest index (21.8%). Likewise, yield components were improved, with grain yield increasing by 44.3% under dual inoculation compared with the control. Furthermore, seed quality traits including 1,000-seed weight (6.2%), seed output (8.4%), amylase activity (9.4%), and protein content (10.3%), were maximized under the combined treatment. Nutrient uptake analysis revealed substantial increases in N, P, and K acquisition, with N uptake rising from 97.4 in control to 133.6 kg-ha⁻¹ with the combined application. Enhanced nutrient assimilation translated into higher N-use efficiency indices (aNUE, pNUE, iNUE, aNRE) in the biotreated variables compared with the control. Soil fertility parameters, including humus, total N, and available nutrient forms, were significantly improved, alongside enriched microbial populations. The combined biofertilizer application resulted in the highest abundance of microbial groups, including ammonifiers (9.0 × 10⁷), oligonitrophils (8.7 × 10⁶), micromycetes (7.5 × 10⁴), and actinomycetes (5.8 × 10⁵), compared with the control. These findings highlight the microbial-enhanced fertilizers as a climate-smart and sustainable nutrient management strategy, offering a pathway to strengthen rice production systems while improving soil health.

KEYWORDS

grain quality, microbial nutrient management, paddy agroecosystem, rice yield, soil fertility, soil microbial activity, sustainable agriculture

1 Introduction

Rice production has increasingly relied on synthetic fertilizers and agrochemicals to sustain yields and meet the demands of a growing population (Primov et al., 2025). The escalating global demand for rice has intensified the overuse of chemical fertilizers, underscoring the urgent need for sustainable, nature-based nutrient management alternatives (Musa et al., 2025). Continuous dependence on chemical inputs has accelerated soil degradation, disrupted nutrient balance, and contributed to environmental contamination (Sultonov et al., 2025). Beyond soil health, excessive fertilizer application exacerbates ecological challenges, including nutrient runoff, greenhouse gas emissions, and destabilization of soil microbial communities. Collectively, these outcomes raise critical concerns regarding the sustainability of conventional rice cultivation systems. Addressing these challenges requires a transition toward nutrient management strategies that are not only productive but also restorative, ensuring resilience of agroecosystems in the face of climate change (Umarov et al., 2025).

Rice (*Oryza sativa* L.) is a highly consumed crop for more than half of the world's population, serving as both the primary source of calories and rural employment. The rice cultivation area in Uzbekistan is approximately 50,000 hectares, with an average yield of 8.3 Mg per hectare (FAOSTAT Statistical Database, 2024). However, water scarcity is reducing the extent of rice production. Therefore, innovative practices are essential to enhance yields and increase output. Although rice production has substantially increased over the last years, domestic demand still is not fully covered due to the increased population. Sustainable rice production depends on nutrient management approaches that maintain soil health, environmental stability and food security. Many resource-saving technologies are being harnessed in the region to rejuvenate supply chain. Understandably, many of these practices were not implemented with a lack of vital characteristics, i.e., adaptability, average yield, and grain quality (Song et al., 2025). Nowadays, biological methods are being widely adopted in crop production worldwide, considering a number of advantages in terms of economic and environmental sustainability. This eco-friendly approach provides essential nutrients, enhance nutrient use efficiency and contribute to more sustainable and resilient crop production systems (Ravshanov et al., 2023).

Advanced biological soil technologies are increasingly being adopted in crop production worldwide, driven by their demonstrated advantages in restoring soil health, improving yields, and supporting environmental sustainability (Rustamova et al., 2023). Unlike conventional input-intensive practices, biological approaches harness naturally occurring microbial and agricultural practices to enhance nutrient cycling, improve soil fertility, and reduce dependence on synthetic agrochemicals (Ospanbayev et al., 2025). Their integration into modern farming systems not only increases production efficiency but also mitigates adverse environmental impacts, thereby contributing to long-term resilience of agroecosystems (Utambetov et al., 2022). As global agriculture faces

mounting pressures from resource constraints and climate change, the shift toward biologically based nutrient management represents a critical pathway for achieving sustainable intensification of crop production.

Although microbial nutrient management practices have been explored, the majority of studies have focused on single microbial biofertilizer or short-term outcomes, without evaluating the performance of biomaterials under diverse field conditions (Jin et al., 2023). Furthermore, empirical evidence linking the integration of microbial compounds with soil nutrient dynamics and regenerative outcomes remains limited. This lack of comprehensive, field-based data limits the development of microbial nutrient management strategies designed to sustain crop productivity while simultaneously restoring soil health (Maksumkhanova et al., 2025). To address this critical research gap, the present study evaluated the effects of biocompounds derived from various soil beneficial bacteria along their integrative effect on the rice growth and yield performance. Based on this rationale, the present study was designed with the following specific objectives: (i) to evaluate the effects of biocompounds on the growth, yield traits and quality indices of rice; (ii) to examine soil nutrient uptake parameters (N, P, K, and Mg) under biocompound applications; and (iii) to identify the most effective regenerative nutrient management strategy capable of sustaining yield while improving soil fertility.

Although a scientific base for the use of microbial compounds in rice production has been established, the implementation of these methods in paddy fields is limited. It was hypothesized that the application of biocompounds would maintain or enhance rice productivity, thereby contributing to regenerative agriculture. Considering these factors, the present study offers a field-based assessment of microbial nutrient management strategies in a widely cultivated rice variety under conditions of declining soil fertility. By evaluating integrated organic approaches within realistic agronomic settings, the research contributes to ongoing efforts to enhance fertilizer use efficiency, strengthen residual soil nutrient reserves, improve ecological resilience, and promote sustainable intensification.

2 Materials and methods

2.1 Study site and soil structure

The field experiment was carried out during 2024–2025 growing seasons at the research station of Rice research institute located in Tashkent region (41°00'N, 64°00'E) in the north-western of Uzbekistan. Continental climate prevails in the region with an average monthly air temperature 0 °C in January and the highest temperature is observed in July with 37 °C. Average yearly rainfall is 200 ± 36 mm and the main part falls in the late autumn till the middle of spring (Figure 1). This amount of precipitation is relatively low compare to crop water requirements and furrow

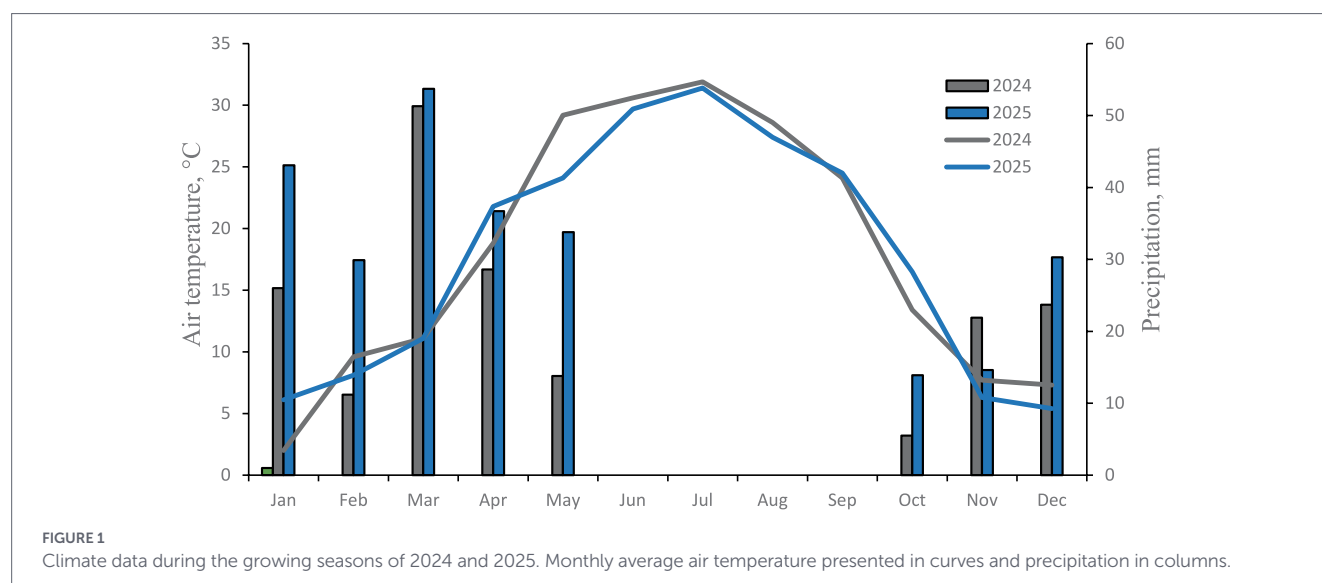


TABLE 1 Physical and chemical properties of soil.

Soil layer, cm	Texture, %			Soil bulk density, g·cm ⁻³	Humus, %	NO ₃ , mg·kg ⁻¹	P ₂ O ₅ , mg·kg ⁻¹	K ₂ O, mg·kg ⁻¹
	Sand	Silt	Clay					
0–30	15.4	68.2	16.4	1.73	1.21	12.2	12.1	216.7
30–60	15.0	68.6	16.4	1.86	1.05	10.3	12.5	180.6
60–90	37.3	46.3	16.4	1.65	0.84	7.12	8.5	240.8

irrigation predominantly used for agricultural production in all over the country (Ibragimov et al., 2021). In January, a cold-weather brings higher humidity reaching slightly more than 80%, whereas the lowest indicator is expected in June with less than 45%.

The selected field's soil was identified as a typical serozem having underground water in 3–4 m deep. The selected field (0.5 ha each) was categorized as a low salinity level based on electrical conductivity (EC: 1.6 ± 0.2 dS·m⁻¹) and pH was neutral, ranging between 7.08–7.39. Silt was predominant in the surface soil horizon, whereas sand share increases in the deeper soil horizons (Table 1). The availability of organic matter is low in orchic horizon (1.07% organic matter, total N 0.11%, P 0.130%, K 0.84%, Mg 6 mg·100 g⁻¹ soil; exchangeable forms 10.7 N-NO₃ mg·kg⁻¹, 29.4 mg·kg⁻¹ P₂O₅, 185 mg·kg⁻¹ K₂O) having a calcic horizon within 90 cm of the surface.

On average, the soil contained 251 ± 3 g sand·kg⁻¹, 615 ± 11 g silt·kg⁻¹, and 166 ± 11 g clay·kg⁻¹, had the cation exchange capacity of 24.2 ± 1 cmol·kg⁻¹, with exchangeable Na percentage of 3.89, and Na absorption ratio of 0.42.

A soil corer (3.5 cm diameter) was used to obtain soil samples of (0–30) cm depth. The soil samples were kept in black polyethylene bags and stored at 4 °C. After being air-dried for 7 days at room temperature (25 °C), these soil samples were ground and sieved to pass thru a 2 mm sieve, and stored in sealable plastic bags until further chemical analysis.

The glass electrode pH meter and Ece electrode were employed to analyze the pH and electrical conductivity (ECe) of the soil paste (1:1) with distilled deionized water, respectively. The micro-Kjeldahl

digestion and distillation method was used for total N content determination (Sáez-Plaza et al., 2013). The ascorbic acid method was used to analyze total phosphorus content in the soil digestate (Murphy and Riley, 1962). The soil extract with Ethylene di-amine-tetra acetic acid was prepared to measure exchangeable Ca and Mg contents by titrating. A flame photometer was hired to test exchangeable Na and K contents. The standard core method was used to calculate soil bulk density (Barik et al., 2014). The standard Bouyoucos hydrometer method was used after removing soil organic matter with 5% H₂O₂ treatment followed by dispersion with 5% Na-hexametaphosphate solution for particle size analysis (Huluka and Miller, 2014).

2.2 Experiment design and data analysis

The field experiment was conducted using a one-factorial randomized design to evaluate the effects of biofertilizer applications on rice growth and yield. Four treatments were established: the control (T1), *Zamin M* (T2), *Simbioz EM-1* (T3), and their combined application (T4). Each treatment was replicated four times, resulting in a total of 16 plots. Each plot measured 40 m² (4 m wide × 10 m long), arranged in a uniform layout to minimize edge effects.

Rice seeds were sown at a seeding norm of 200 kg·ha⁻¹ during the second half of May in both years of the study. Phosphorous and potassium types of chemical fertilizers were applied at norms of 140 and 70 kg·ha⁻¹·yr⁻¹, respectively, in all plots of the experiment. Rice cultivation measures such as irrigation, pest and weed control were conducted based on the recommended conventional management practices.

Rice cultivation in Uzbekistan has traditionally relied on flood irrigation, where fields are continuously supplied with water through canal systems to maintain shallow flooding during the growing season. In this experiment, irrigation was managed according to local agronomic norms: water was applied uniformly across all plots, with a total of 25,000 m³ per ha supplied to maintain consistent soil moisture conditions. This ensured that each treatment received equal water input, thereby eliminating irrigation variability as a confounding factor. While flood irrigation is effective for weed suppression and crop establishment, it is highly water-intensive and contributes to soil salinity and evaporative losses. Including this detail provides transparency and situates the study within the prevailing regional practice of rice production in Uzbekistan.

2.3 Bacterial fertilizers

The microbial inoculant *Zamin M* was obtained from the culture collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan. The formulation consisted of *Azotobacter chroococcum* at a concentration of 5×10^9 CFU/mL, prepared as a liquid suspension. This single-strain inoculant was applied at a rate of 2 L·ha⁻¹, diluted in irrigation water to ensure uniform distribution across the plots. The high cell density was selected to promote effective colonization of the rice rhizosphere and to enhance biological nitrogen fixation under arid field conditions. The detailed specification of microbial strain and concentration ensures reproducibility and transparency of the experimental design.

The microbial inoculant *Simbioz EM-1* produced by the Mufas company located in Tashkent, Uzbekistan. This biocompound was applied as a liquid suspension containing a consortium of beneficial soil microorganisms. The formulation included *Azotobacter chroococcum* (1.2×10^8 CFU/mL), *Bacillus subtilis* (9.5×10^7 CFU/mL), and *Lactobacillus plantarum* (8.7×10^7 CFU/mL). In addition, the inoculant contained *Chlorella vulgaris* (1.0×10^6 cells/mL), which served as an active component contributing to nutrient cycling and microbial stability. Organic acids (lactic and humic acids, 0.05% w/v) were included as carriers and stabilizers, enhancing microbial viability during storage and application. The inoculant was applied at a rate of 2 L·ha⁻¹, diluted in irrigation water, to ensure uniform distribution across the plots. This detailed composition and concentration information allows reproducibility of the experiment and clarifies the functional role of each component in the formulation.

Rice seeds of the *Iskandar* variety were obtained from the Rice Research Institute, Tashkent Region, Uzbekistan. The *Iskandar* rice variety is a mid-ripening Uzbek cultivar tested under different irrigation regimes. It performs best with a 15 cm water layer, giving about 77% seed yield and 72% milled rice yield. This cultivar is particularly valued for its stress tolerance, consistently exhibiting stable yield performance and good quality grains. This rice variety “*Iskandar*” has shown a yield potential of about 5.5–8.0 tons per hectare under favorable irrigated conditions. Prior to experimentation, seeds were sorted to remove broken, undersized, and visibly infected grains. The selected seeds were surface-sterilized using a solution composed of 75 mL chloride and 25 mL distilled water for 2–3 min, followed by five successive rinses with sterile distilled water. Subsequently, seeds were inoculated with biofertilizers for 30 min in accordance with standard procedures.

Seeds were manually sown in water-filled plots with an approximate water depth of 15 cm. Strict protective measures were implemented to prevent cross-contamination among different biotreatment groups. The biofertilizers are applied mainly through seed treatment during the planting process. This practice improves soil microbial activity and nutrient cycling. During the vegetation period, biofertilizers were additionally applied twice directly to designated plots according to the experimental design.

2.4 Plant analysis

During the growing period, the parameters of seed germination, shoot/root fresh and dry weight, plant height, and a number of productive shoots were recorded. Dry weights of shoot and root were determined after drying in an oven for 1 day at 70 °C. Rice plants were harvested according to their maturity time after 115–120 days of seed sowing and then were used to estimate the yield based on the plot yield and grain quality indicators. The field trial was designed to reflect local farming conditions and smallholder practices, thereby enhancing the practical relevance and transferability of the findings.

Plant samples randomly were collected immediately before harvest were washed by spraying with distilled water, oven-dried at 65 °C for 72 h, ground to pass through a 0.5 mm sieve, and stored in sealable plastic bags prior to analysis. While total N content was determined by the micro-Kjeldahl method (Chapman and Pratt, 1961), total P content in acid digestate was determined spectrophotometrically by following the ascorbic acid method (Murphy and Riley, 1962). Total Ca, Mg, and K contents were determined in diluted acid digestate by using the flame photometry procedure. For the calculation of N, P, K, Ca and Mg uptake, the N, P, K, Ca and Mg concentrations were multiplied with the respective shoot dry-weights of the rice.

Amylose content in rice seeds was determined by the standard method with some modifications (Juliano et al., 1972). Total protein content was detected by the Kjeldahl method according to the AOAC International (Latimer, 2016).

2.5 Nitrogen-use efficiency (NUE)

2.5.1 Agronomic nitrogen-use efficiency (aNUE)

This was determined using the following equation (Novoa and Loomis, 1981) exhibiting on the yield increase due to N fertilization (Y_f) as compared to the control (Y_0) treatment:

$$\text{aNUE}(\text{kg} \cdot \text{kg}^{-1}) = \frac{Y_f - Y_0}{\text{N fertilization}}$$

2.5.2 Physiological nitrogen-use efficiency (pNUE)

This was evaluated by a method developed by Isfan (1990) based on the increase in rice grain yield per unit of increased N uptake:

$$\text{pNUE}(\text{kg} \cdot \text{kg}^{-1}) = \frac{Y_f - Y_0}{\text{TNU}_f - \text{TNU}_0}$$

where, the TNU_f and TNU_0 are the N uptake in N fertilized- and the control plots, respectively.

2.5.3 Internal nitrogen-use efficiency (INUE)

This was calculated using a method by Witt et al. (1999) depending rice yield increase per unit tissue N concentration.

$$iNUE(g\cdot g^{-1}) = \frac{\text{Grain yield}}{\text{Tissue N concentration}}$$

2.5.4 Apparent nitrogen recovery efficiency (aNRE)

This was calculated by the following equation by Dilz (1988) based on the increased total N uptake in response to N fertilization:

$$aNRE(\%) = \left[\frac{TNU_f - TNU_0}{N_{\text{fertilization}}} \right] * 100$$

where, the TNU_f and TNU_0 are the N uptake in N fertilized- and the control plots, respectively.

2.6 Statistical procedures

Data were analyzed for statistical significance using the ANOVA (Cropstat program). Mean comparisons were conducted by Student's *t*-test using a least significant difference (LSD) test ($p = 0.05$).

3 Results

3.1 Effect of bioproducts on rice growth attributes

The applied bioproducts significantly enhanced seed germination, plant height, biomass accumulation, and leaf area index, demonstrating consistent efficiency compared with the untreated control (Table 2). Germination percentage increased from 59.6% in the control to 71.5 and 74.6% under Zamin and Simbioz EM-1 treatments,

respectively. The most pronounced germination effect was observed under the combined treatment, resulting in a 30.2% increase over the control. The combined application enhanced seed vigor and establishment. Plant height followed a similar trend, increasing from 97.7 cm in the control to 105.0 and 111.6 cm under Zamin and Simbioz EM-1 treatments, respectively. These values were statistically significantly higher than the control (97.7 cm). The tallest plants were recorded in the combined treatment (120.3 cm), significantly exceeding the control (97.7 cm).

Fresh and dry biomass accumulation also increased, with fresh weight rising from 18.2 g to 26.6 g and dry weight from 7.3 g to 9.6 g. The combined application produced the highest values (26.6 g and 9.6 g), corresponding to a 46.2% increase in fresh weight and a 31.5% increase in dry weight. Simbioz EM-1 followed closely (25.9 g and 9.1 g), while Zamin M showed the lowest effect (24.0 g and 8.6 g), with statistically significant differences among treatments.

A similar trend was observed for leaf area indices. The highest value of leaf area per plant was found under the combined biotreatment application (193.6 cm²) which increased by 18.3% as compared to the control (163.7 cm²). This expansion reflects enhanced photosynthetic capacity, contributing to stronger canopy development and more efficient resource capture in rice systems. Statistical analysis confirmed that these differences were significant at the 5% level, with low coefficients of variation (3.8–5.0%) indicating experimental reliability. While single inoculants (Zamin M and Simbioz EM-1) provided measurable improvements over the control, their combined application consistently produced the highest values across all parameters. This suggests a synergistic effect of dual inoculation, likely due to enhanced microbial colonization, nutrient uptake, and root development.

Overall, the findings demonstrate that biofertilizers, particularly when applied in combination, represent a practical and climate-smart approach to strengthening rice productivity and improving soil fertility.

3.2 Effect on yield components and seed quality

During the 2024 and 2025 growing seasons, the application of biofertilizers significantly influenced economic, biomass, and total yield parameters as well as HI (Table 3). Zamin M increased economic, biomass, and total yields and HI by 15.6, 8.5, 30.4, and 8.8%, respectively, compared with the control. Consistent improvements were also recorded under the Simbioz EM-1 treatment, with increases of 18.4, 9.6,

TABLE 2 Effects of biofertilizers on growth parameters of rice (averaged across 2024 and 2025 growing seasons).

Treatments	Germination (%)	Height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ² /per plant)
Control	59.6d	97.7d	18.2d	7.3d	163.7c
Zamin M	71.5c	105.0c	24.0c	8.6c	187.1bc
Simbioz EM-1	74.6b	111.6b	25.9b	9.1b	188.4b
Dual application	77.6a	120.3a	26.6a	9.6a	193.6a
LSD _{0.05}	2.3	4.2	0.54	0.45	3.4
CV (%)	4.8	4.6	3.8	5.0	4.6

Means separated by same lower case letter (a and d) in each column were not significantly different between treatments at $p \leq 0.05$.

TABLE 3 The effects of the applied biocompounds on total, biomass, economic, total yield and HI of rice (averaged across 2024 and 2025 growing seasons).

Treatments	Total	Biomass	Grain	HI
		Yield (mg·ha ⁻¹)		(kg·kg ⁻¹)
Control	13.91b	9.4b	4.51d	0.32c
Zamin M	16.08ab	10.2ab	5.88c	0.37b
Simbioz EM-1	16.47ab	10.3a	6.17b	0.37b
Dual application	16.71a	10.2ab	6.51a	0.39a
LSD _{0.05}	1.3	0.32	0.24	0.11
CV (%)	3.8	6.6	3.8	3.6

HI, harvest index indicator of rice. Means separated by same lower case letter (a and d) in each column were not significantly different between treatments at $p \leq 0.05$.

TABLE 4 The effects of the applied biocompounds on rice seed quality characteristics (averaged across 2024 and 2025 growing seasons).

Treatments	Weight of 1,000 seeds (g)	Seed output (%)	Amylase (%)	Protein (%)
Control	32.2c	60.5d	19.2c	6.8b
Zamin M	33.4bc	63.1c	20.7bc	7.0ab
Simbioz EM-1	33.6b	64.2b	20.8b	7.3ab
Dual application	34.2a	65.6a	21.0a	7.5a
LSD _{0.05}	0.3	0.42	0.14	0.51
CV (%)	2.8	6.6	3.8	4.0

Means separated by same lower case letter (a and d) in each column were not significantly different between treatments at $p \leq 0.05$.

36.8, and 11.8% relative to the control. The effect of the applied biofertilizers was most pronounced under the dual application, which produced greater increases in economic, biomass, and total yield parameters as well as the harvest index compared with all other treatments. These values were 20.1, 8.5, 44.3, and 11.0% higher than those observed in the control.

Notably, the total yield increased by 20.2%, from 13.91 Mg·ha⁻¹ in the control to 16.71 Mg·ha⁻¹ under dual application, while biomass yield rose from 9.4 Mg·ha⁻¹ to 10.2 Mg·ha⁻¹. Regarding the economic yield, most pronounced improvement was found under the combined treatment, exhibiting 44.3% increase compared to the control indices. The differences between the single treatments Zamin M (5.88 Mg·ha⁻¹) and Simbioz EM-1 (6.17 Mg·ha⁻¹) were statistically significant ($p > 0.05$). Although Simbioz EM-1 produced higher total and biomass yields compared with Zamin M, these increases did not reach statistical significance.

The harvest index also improved, with the control recording 0.32, whereas Simbioz EM-1 and Zamin M reached 0.37. The dual application significantly surpassed all other treatments, reflecting more efficient partitioning of assimilates into grain.

Statistical analysis confirmed that these differences were significant at the 5% level, and the relatively low coefficients of variation (3.6–6.6%) indicate strong experimental reliability. While single inoculants such as Zamin M and Simbioz EM-1 enhanced yield parameters compared to the control, their combined application consistently produced the highest values across all measured traits. This suggests a synergistic effect of dual inoculation, likely driven by improved microbial colonization, nutrient uptake, and root development (see Table 4).

The application of biofertilizers exerted a significant influence on rice seed quality traits averaged across the 2024 and 2025 growing seasons. Dual application consistently produced the highest values across all measured parameters. The 1,000-seed weight increased from 32.2 g

in the control to 34.2 g under dual application, representing a statistically significant improvement (LSD = 0.3, $p < 0.05$). Single applications of Zamin M (33.4 g) and Simbioz EM-1 (33.6 g) also enhanced seed weight compared with the control, though to a lesser extent.

Seed output followed a similar trend, rising from 60.5% in the control to 65.6% under dual application. Zamin M and Simbioz EM-1 improved seed output to 63.1 and 64.2%, respectively, with all treatments significantly outperforming the control (LSD = 0.42, $p < 0.05$).

Biochemical quality traits were likewise enhanced. Amylase content increased from 19.2% in the control to 21.0% under dual application, while single treatments yielded 20.7% (Zamin M) and 20.8% (Simbioz EM-1). Protein concentration rose from 6.8% in the control to 7.5% under dual application, with single treatments also showing significant gains (7.0 and 7.3%).

The relatively low coefficients of variation (2.8–6.6%) confirm the reliability and consistency of the experimental results.

The synergistic effect of combining biofertilizers likely promotes microbial colonization and nutrient assimilation, resulting in improved seed weight, output, and nutritional composition. The dual application strategy emerges as a practical and climate-smart approach to strengthening yield outcomes and promoting sustainable rice cultivation.

Overall, the findings demonstrate that biofertilizers, particularly when applied in combination, significantly enhance rice productivity and seed quality. These findings demonstrate that biofertilizer application, particularly in dual form, enhances both yield and seed quality traits in rice.

3.3 Nutrient dynamics

The application of biofertilizers significantly enhanced the uptake of macronutrients (N, P, K) in rice compared to the control group values. N uptake increased from 97.4 kg·ha⁻¹ in the control to

105.9 kg-ha⁻¹ under the Zamin M treatment, representing a 37% increase. Simbioz EM-1 demonstrated further improvement, with a 17.5% increase relative to the control. The highest N uptake was recorded under the combined treatment (133.6 kg-ha⁻¹), representing a 37.2% increase compared to the control.

P uptake showed a similar trend, with the highest values recorded under the dual application (37.9 kg-ha⁻¹), followed by Simbioz EM-1 (36.1 kg-ha⁻¹) and both statistically superior to the control (27.4 kg-ha⁻¹). K uptake was maximized under dual application (127.0 kg-ha⁻¹), exceeding the control by nearly 20 kg-ha⁻¹ (see Table 5).

Ca uptake remained relatively stable across treatments, however, the applied treatments showed statistically significant differences compared to the control. In terms of Mg uptake, sole or combination of biocompounds had almost similar values during both the study years (means sharing the same letter). This suggests that biofertilizer application primarily influences N, P, and K dynamics, while Ca and Mg uptake is less responsive under the tested conditions. The relatively low coefficients of variation (CV < 5%) confirm the reliability of the observed treatment effects.

The microbial biofertilizers, particularly when applied in combination, substantially improve nutrient acquisition in rice, with the strongest effects observed for N and P. This highlights their potential role in reducing chemical fertilizer dependency while sustaining crop productivity.

Across two growing seasons, biofertilizer application markedly improved NUE parameters in rice. aNUE increased from 39.7 kg/kg in the control to 48.3 kg/kg under dual application, reflecting enhanced yield response per unit of applied N. Similar patterns were followed in terms of pNUE and iNUE, where dual application

consistently outperforming single treatments. Likewise, aNRE rose from 31.5% in the control to 49.5% under dual application, indicating improved nitrogen uptake and utilization by the crop (see Table 6).

The LSD values confirm that these differences are statistically significant, while the relatively low CV values (2.8–8.0%) demonstrate experimental precision. Among single applications, Simbioz EM-1 consistently outperformed Zamin M, suggesting stronger microbial activity or compatibility with rice rhizosphere under open field conditions. Dual biofertilizer application optimizes nitrogen-use efficiency in rice, particularly under stress-prone saline soils. This improvement in both agronomic and physiological efficiency underscores the potential of integrated biofertilizer strategies to enhance sustainability and resilience in rice production systems.

The combined evidence from rice trials demonstrates that biofertilizers especially when applied in dual combinations—significantly improve nutrient uptake and nitrogen-use efficiency. These findings support the integration of biofertilizers into nutrient management programs for major crops in continental climates, offering a pathway toward reduced reliance on synthetic fertilizers, improved soil health, and enhanced crop resilience under challenging conditions such as salinity.

3.4 Soil biological activity

The results in Table 7 presented microbial populations in the rice rhizosphere. At the start of the experiment, microbial counts were balanced but modest. The applied biocompounds significantly increased a number of soil beneficial bacteria in the rice rhizosphere.

TABLE 5 The effects of the applied biocompounds on total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) uptake of rice.

Treatments	N	P	K	Ca	Mg
	(kg-ha ⁻¹)				
Control	97.4d	27.4c	107.4d	16.8b	4.8a
Zamin M	105.9c	34.4b	115.3c	17.5a	4.9a
Simbioz EM-1	114.6b	36.1ab	121.0b	17.7ab	4.8a
Dual application	133.6a	37.9a	127.0a	17.8a	4.8a
LSD _{0.05}	8.3	2.22	5.14	0.4	3.4
CV (%)	4.8	3.6	3.8	4.0	2.6

Means separated by same lower case letter (a and d) in each column were not significantly different between treatments at $p \leq 0.05$.

TABLE 6 Effects of the applied biofertilizers on nitrogen-use efficiency in rice (averaged across 2024 and 2025 growing seasons).

Treatments	aNUE	pNUE	iNUE	aNRE
	(kg-kg ⁻¹)		(g-g ⁻¹)	(%)
Control	39.7d	41.8d	44.3d	31.5d
Zamin M	43.1c	46.8c	46.5c	37.0c
Simbioz EM-1	45.6b	50.2b	48.1b	42.5b
Dual application	48.3a	53.6a	50.6a	49.5a
LSD _{0.05}	2.3	2.23	1.14	2.1
CV (%)	2.8	6.6	3.8	8.0

aNUE, agronomic nitrogen-use efficiency; pNUE, physiological nitrogen-use efficiency; iNUE, internal nitrogen-use efficiency; aNRE, Apparent nitrogen recovery efficiency. Means separated by same lower case letter (a and d) in each column were not significantly different between treatments at $p \leq 0.05$.

TABLE 7 Quantity of microorganisms in the rice rhizosphere as affected by the applied biocompounds (averaged across 2024 and 2025 growth seasons).

Treatments	Ammonifiers	Oligonitrophils	Micromycetes	Actinomycete
At the beginning of the experiment				
	7.5 × 10 ⁶ c	4.1 × 10 ⁶ c	5.5 × 10 ⁴ c	4.2 × 10 ⁵ c
At the vegetation period				
Control	5.2 × 10 ⁶ d	3.5 × 10 ⁵ d	3.5 × 10 ⁴ d	3.5 × 10 ⁴ d
Zamin M	4.8 × 10 ⁷ c	4.7 × 10 ⁶ c	5.7 × 10 ⁴ c	4.5 × 10 ⁵ c
Simbioz EM-1	6.1 × 10 ⁷ b	6.7 × 10 ⁶ b	6.7 × 10 ⁴ b	5.5 × 10 ⁵ b
Dual application	9.0 × 10 ⁷ a	8.7 × 10 ⁶ a	7.5 × 10 ⁴ a	5.8 × 10 ⁵ a
LSD _{0.05}	1.9	1.2	0.6	0.2
CV (%)	4.7	3.4	4.3	3.7

Means of three replications (*n* = 3) separated by lower case letters (a and d) in each column are significantly different at *P* ≤ 5%.

TABLE 8 Nutrient content in soil as affected by the applied biocompounds (averaged across 2024 and 2025 growth seasons).

Treatments	Soil horizon (cm)	Total forms (%)			Available forms (mg·kg ⁻¹)		
		Humus	N	P	NO ₃	P ₂ O ₅	K ₂ O
At the beginning of the experiment							
	0–30	0.85b	0.078b	0.168b	3.23c	24.24b	265c
At the end of the vegetation period							
Control	0–30	0.81c	0.077b	0.165c	2.84c	23.94b	261c
Zamin M	0–30	0.85b	0.079b	0.168b	3.11c	25.26b	270b
Simbioz EM-1	0–30	0.89b	0.080b	0.170a	4.15b	24.78b	276b
Dual application	0–30	0.92a	0.086a	0.172a	5.74a	27.24a	286a
LSD _{0.05}		0.3	0.0041	0.014	1.14	1.16	5.6
CV (%)		2.8	3.6	3.8	5.6	3.4	4.5

Means of three independent replications (*n* = 3) separated by lower case letters (a and c) in each column are significantly different at *p* ≤ 5%.

By the end of the vegetation period, the dual application treatment yielded the highest abundance of all microbial groups: ammonifiers (9.0 × 10⁷), oligonitrophils (8.7 × 10⁶), micromycetes (7.5 × 10⁴), and actinomycetes (5.8 × 10⁵). These increases suggest enhanced rhizosphere activity, contributing to nutrient mineralization and improved plant nutrient uptake. Microbial population dynamics further support this conclusion. Statistical analysis confirmed significant differences among treatments, with low variability across replications.

The application of Simbioz EM-1 and Zamin M positively influenced the microbial community in the rice rhizosphere, with ammonifier populations of 6.1 × 10⁷ and 4.8 × 10⁷, respectively. All amended treatments significantly exceeded the control, with clear statistical differences observed among treatments. A similar trend was recorded for Oligonitrophil, Micromycete, and Actinomycete populations. The maximum microbial abundance was achieved under the dual application.

The results presented in Table 8 demonstrate clear effects of the applied biocompounds on soil nutrient dynamics. Soil analysis revealed significant differences in nutrient concentrations among treatments (*p* < 0.05). At the beginning of the experiment, baseline values of humus, N and P were moderate, while available nutrient forms such as NO₃, P₂O₅, and K₂O were relatively stable. During the vegetation period, significant differences emerged among treatments. The dual application of biocompounds consistently produced the

highest values across all measured parameters, including humus (0.92%), total N (0.086%), NO₃ (0.172 mg·kg⁻¹), P₂O₅ (5.74 mg·kg⁻¹), and K₂O (27.24 mg·kg⁻¹). N content ranged from 0.077% in the control to 0.086% in the dual treatment, exhibiting the highest value with substantial difference compared to the control. This was followed by Simbioz EM-1 (0.080%) and Zamin M (0.079%), recording significantly higher N levels than the control groups. These improvements were statistically significant, as confirmed by LSD values, with coefficients of variation below 6%, indicating reliable experimental precision. The findings highlight the synergistic effect of combined biocompounds in enhancing soil fertility.

Overall, the data indicate that dual application of biocompounds is superior to single applications or control treatments in improving both microbial activity and soil nutrient status. The consistency of results across two growing seasons (2024–2025) strengthens the reliability of these findings. This evidence supports the role of microbial consortia in sustainable nutrient management, offering a promising approach to enhance soil fertility and rice productivity under field conditions.

Humus content increased with biocompound application, with the dual treatment maintaining the highest value (0.92%), followed by Simbioz EM-1 (0.89%) and Zamin M (0.85%), both of which were statistically higher than the control.

Likewise, P concentrations varied between 0.165% in the control to 0.172% in dual application as compared 0.168% at the beginning of

the experiment. Dual application significantly exceeded all other treatments, while Simbioz EM-1 (0.89%) showed higher P content than Zamin M (0.85%), both of which were statistically higher compared to the control.

A similar trend was recorded for observed available forms of soil nutrients. The highest concentration NO_3 was observed under dual treatment (5.74 $\text{mg}\cdot\text{kg}^{-1}$), followed by Simbioz EM-1 application (4.15 $\text{mg}\cdot\text{kg}^{-1}$) and Zamin M (3.11 $\text{mg}\cdot\text{kg}^{-1}$). These were significantly greater than the control (2.84 $\text{mg}\cdot\text{kg}^{-1}$), which recorded the lowest values.

These findings indicate that integrating microbial compounds can enhance soil fertility, particularly N and P in rice grown fields.

4 Discussion

4.1 Growth and yield responses to biocompounds

The findings of this study clearly demonstrate that biofertilizer application, particularly in dual combinations, exerts a significant positive influence on rice growth, yield, seed quality, nutrient dynamics, and soil fertility. Across two growing seasons, consistent improvements were observed in germination percentage, plant height, biomass accumulation, and leaf area index.

The application of bioproducts substantially improved rice growth attributes compared to the untreated control, confirming their effectiveness under field conditions. While single inoculants (Zamin M and Simbioz EM-1) enhanced germination, plant height, biomass, and leaf area, their combined application consistently produced the highest values, indicating a synergistic interaction. This synergism likely reflects improved microbial colonization, nutrient uptake, and root development, which collectively strengthened seed vigor and photosynthetic capacity. Overall, the findings highlight dual inoculation as a climate-smart strategy for enhancing rice productivity and sustaining soil fertility.

The dual application proved particularly effective, resulting in the highest grain yield (6.51 $\text{Mg}\cdot\text{ha}^{-1}$), biomass yield (16.71 $\text{Mg}\cdot\text{ha}^{-1}$), and harvest index (0.39). Such performance reflects the balanced macro- and micronutrient composition of the applied biocompounds, their capacity to stimulate microbial activity, thereby enhancing nutrient mineralization and uptake throughout the growth cycle. Comparable responses have been documented in long-term integrated nutrient management systems, where compost application increased rice yield and nutrient recovery (Israilov et al., 2025).

Single treatments also significantly increased yields compared to the control. Grain yield was enhanced by 36.8% with Simbioz EM-1 and by 30.4% with Zamin M. Consistent with the observations of Somboon et al. (2024), bacterial fertilizers derived from beneficial soil microbes not only enhanced crop yield but also improved soil organic carbon and cation exchange capacity. The positive yield response in the present study is most plausibly attributed to stimulated soil biological activity, which increased nutrient availability and strengthened the functional capacity of the rhizosphere.

Across all amended treatments, total biomass yield was significantly higher than the control (9.4 $\text{Mg}\cdot\text{ha}^{-1}$). This rice characteristic reached 10.2 $\text{Mg}\cdot\text{ha}^{-1}$ with dual bioapplication, while single application with Simbioz EM-1 and Zamin M exhibited almost similar values showing 10.3 and 10.2 $\text{Mg}\cdot\text{ha}^{-1}$. However, total biomass partitioning ratios differ significantly among treatments, indicating that yield gains were primarily due to increased total biomass rather than altered allocation patterns.

In agreement with these outcomes, Shang et al. (2025) demonstrated that integrating organic fertilizers sustained organic matter partitioning while enhancing grain accumulation. The significant variation in HI among treatments indicates that yield gains were primarily driven by altered assimilate allocation rather than mere increases in total biomass. This suggests that organic amendments modified the intrinsic source-sink balance and yield architecture, thereby enhancing plant growth capacity through improved nutrient availability, which resulted in higher grain yield rather than biomass accumulations. Such outcomes align with regenerative nutrient management systems that strengthen resource capture and physiological vigor while maintaining partitioning efficiency (Banayo et al., 2012; Mohamed et al., 2022).

Seed quality traits were similarly enhanced under biofertilizer treatments. These improvements were accompanied by superior grain quality, including the greatest 1,000-grain weight (34.2 g), seed output (65.6%), and elevated amylase (21.0%) and protein content (7.5%). Notably, the combined treatment increased total yield by 44.3% compared with the control, underscoring its potential to substantially boost productivity and quality. Improvements in seed weight, output, and biochemical quality (amylase and protein content) further highlight the role of microbial inoculants in enhancing both quantitative and qualitative aspects of rice production.

While single inoculants (Zamin M and Simbioz EM-1) provided measurable benefits for rice vegetative and generative development compared with the control, their combined application consistently produced the highest values, indicating a synergistic effect. In agreement with this study, Nazma et al. (2023) emphasized that effective beneficial bacterial consortia within plant rhizosphere symbioses capable of suppressing infections and promoting growth represents a more practical, rapid, and cost-effective strategy for sustainable crop improvement. This synergy is likely attributable to enhanced microbial colonization, improved nutrient uptake, and stronger root development, which collectively strengthened plant establishment and vigor.

4.2 Nutrient dynamics and soil fertility

Nutrient uptake dynamics revealed that biofertilizers substantially improved nitrogen, phosphorus, and potassium acquisition, with the strongest effects recorded under dual application. These improvements translated into higher aNUE, pNUE, iNUE, and aNRE, confirming that the applied biofertilizers had beneficial effect only enhance nutrient availability but also optimize their utilization. These findings provide compelling quantitative evidence that the applied biocompounds enhance nutrient uptake and crop productivity. The gradual nutrient release through organic matter mineralization appears to synchronize more effectively with crop demand than the rapid-release profile of

inorganic fertilizers, thereby minimizing nutrient losses via leaching and volatilization. Such efficiency gains are critical for reducing reliance on synthetic fertilizers and mitigating environmental impacts (Wang et al., 2024).

The combined application of biocompounds approach surpassed other used single treatments in several parameters, demonstrating improved nutrient use efficiency. This is consistent with results from Khan (2018), where biofertilizers reduced N losses and boosted N use efficiency, improved microbial community stability, leading to yield benefits.

Soil fertility parameters further validated the benefits of biofertilizer application, facilitating relationships among soil, plants and microbes that regulate the movement of micronutrients. The dual biotreatments produced the highest values of humus, total nitrogen, and available nutrient forms (NO_3 , P_2O_5 , K_2O), alongside significant increases in microbial populations such as ammonifiers, oligonitrophils, micromycetes, and actinomycetes. These results suggest that biofertilizers stimulate rhizosphere activity, accelerate nutrient mineralization, and foster a more diverse and resilient microbial community, leading to enhanced soil fertility.

The biological interventions used in this study offers substantial environmental and soil health benefits. The applied biocompounds not only contributed to nutrient supply but also improved soil properties critical for long-term crop productivity, showing to be particularly effective in improving nutrient availability. In the present finding, soil nutrient analysis further underscores the regenerative potential of integrated amendment–fertilizer strategies. The combined bioapplication resulted in the highest N and P concentrations, reflecting its capacity to supply these nutrients in available forms through continuous organic matter mineralization. This sustained nutrient availability aligns with regenerative agriculture principles by enhancing nutrient cycling and reducing reliance on external synthetic inputs (Liu et al., 2022).

The differential nutrient responses observed among biotreatments can be explained by the unique physicochemical characteristics of each biomaterial and their interactions exhibited a pronounced effect. The applied biocompounds with its balanced macro- and micronutrient composition and high microbial activity, likely promoted rapid mineralization and continuous N and P release throughout the season. These used biocompounds characterized by high microbial population, may have moderated nutrient release by adsorbing soluble nutrients and providing microhabitats for beneficial microbes, leading to gradual nutrient availability and enhanced soil fertility.

Elevated N and P concentrations in the soil of the biotreated plots suggest that integrating biofertilizers with targeted supplementation enhances the retention of this mobility-prone nutrient, contributing to long-term soil fertility. Such improvements in soil nutrient status indicate not only immediate crop benefits but also a progressive build-up of nutrient reservoir, fostering resilience in subsequent cropping cycles. By replenishing key macronutrients and enhancing nutrient-use efficiency, these practices restore soil function and align with regenerative agriculture's goals of building soil health, closing nutrient loops, and sustaining productivity with reduced environmental impact.

Microbial dynamics constitute a critical pathway through which the mobilization of soil nutrients is regulated, ultimately enhancing crop productivity. Shan et al. (2023) demonstrated that biofertilizers increased bacterial diversity, enriched beneficial taxa,

and suppressed pathogenic populations, outcomes directly associated with yield gains. Such shifts in microbial community structure provide substrates and ecological niches for beneficial microbes, thereby strengthening nutrient cycling efficiency. Consistently, Habibi et al. (2023) reported that microbial nutrient management improved nutrient uptake efficiencies through effective microbial colonization, robust root establishment, and early nutrient uptake. This practice is believed to accelerate microbial activity in the rhizosphere, enhance nutrient supplementation, and contribute to improved soil fertility.

4.3 Environmental and sustainability implications

The integration of biofertilizers, as demonstrated in this study, offers a practical pathway toward regenerative agriculture in rice-based systems. These outcomes provide a strong case for scaling such integrated nutrient management practices in rice-based systems, especially in regions facing soil degradation, nutrient imbalance, and environmental constraints. Regenerative agriculture emphasizes building soil health, enhancing biodiversity, and improving ecosystem functions while maintaining economic viability (Khaitov et al., 2025). The observed improvements in growth, yield, and biomass particularly under bio applications are consistent with the regenerative principle of working with natural processes to restore soil organic matter and nutrient cycling. The applied biocompounds provided diverse functional benefits: nutrient supply, improved soil structure, enhanced microbial habitats, and, long-term carbon sequestration potential. These outcomes are aligned with global regenerative agriculture goals of reducing synthetic input dependency, increasing resilience to climatic variability, and enhancing ecosystem services.

Despite the widespread adoption of biofertilizers, limited research has systematically evaluated rice performance under regenerative nutrient regimes, particularly when biofertilizers are applied in combination (Marzouk et al., 2023). Most existing studies in arid environments have concentrated on short-term yield gains, with comparatively little attention given to the long-term processes of soil regeneration and ecological restoration (Sher et al., 2024).

Achieving this goal necessitates scalable, field-based approaches and innovative solutions that serve as viable pathways toward low-carbon, climate-resilient farming systems. Integrating innovative nutrient management practices into rice production system is therefore critical not only for food security but also for the sustainability of the socioeconomic well-being of smallholders (Karimov et al., 2025). These innovations grounded in nature-based solutions such as soil carbon sequestration, regenerative agriculture, and ecosystem-based adaptation, enhance the management of climate risks across agricultural landscapes. The wide-scale use of these approaches will promote cost-effective renewable energy interventions, improving nutrient availability while simultaneously input efficiency (Khaitov et al., 2024). Current evidence indicates that restoring balance between ecosystem services requires a more integrated approach to managing land, water, and biodiversity. By advancing sustainable practices such as agroecology, precision agriculture, diversification, and biological interventions, this study contributes to resilient production systems that restore soil health, enhance crop productivity and protect ecosystems.

As demonstrated in this study, the applied biological interventions form an integral component of broader nature-based solutions, contributing to habitat protection and expansion, species diversity, ecosystem connectivity, and the overall health of native ecosystems. Therefore, the demonstrated microbiological nutrient management emerges not only as an agronomic advancement but also as a globally significant sustainability strategy. It addresses soil degradation, enhances productivity, reduces environmental impact, and contributes to climate resilience making it a valuable model for the broader framework of regenerative agriculture. In future research, it will be essential to conduct extended multi-season trials to assess how combined biofertilizer applications influence soil organic matter restoration, microbial diversity, and carbon sequestration, while simultaneously enhancing rice tolerance to drought, salinity and climate stress.

5 Conclusion

The application of biofertilizers (Zamin M and Simbioz EM-1) significantly enhanced rice growth, yield performance, and soil nutrient status. The greatest improvements were achieved under the combined treatment, which produced the highest grain yield ($6.51 \text{ Mg}\cdot\text{ha}^{-1}$) and total biomass yield ($16.71 \text{ Mg}\cdot\text{ha}^{-1}$). These values represent a 44.3% increase in grain yield and a 20.1% increase in total biomass compared with the control. Single treatments also significantly improved grain yield traits, with increases of 36.8% under Simbioz EM-1 and 30.4% under Zamin M compared with the control.

The results showed that maximum N, P, and K uptake of 133.6, 37.9, and $127.0 \text{ kg}\cdot\text{ha}^{-1}$, respectively, was achieved under the dual biofertilizer application, outperforming other treatments. Soil analysis further revealed enhanced nutrient availability and microbial activity in biotreated plots, with the most pronounced effects observed under the combined application.

Overall, integrating biofertilizers significantly enhanced rice yield while improving soil health, fostering resilience and reducing dependence on synthetic inputs. These findings underscore that this eco-friendly pathway plays a vital role in regenerative nutrient management within rice systems, while simultaneously fostering sustainable agriculture and driving climate-smart transformation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SZ: Writing – review & editing, Methodology, Writing – original draft, Investigation. KD: Formal analysis, Writing – review & editing, Writing – original draft, Data curation, Software. AB: Writing – review & editing, Formal analysis, Validation, Methodology, Writing – original draft. PB: Writing – original draft, Supervision, Writing – review & editing, Formal analysis, Data curation. AA: Visualization, Project administration,

Writing – review & editing, Data curation, Writing – original draft. ST: Resources, Writing – original draft, Formal analysis, Investigation, Writing – review & editing. AM: Formal analysis, Writing – review & editing, Data curation, Writing – original draft, Investigation. GA: Writing – review & editing, Investigation, Writing – original draft, Data curation, Formal analysis. AT: Supervision, Formal analysis, Writing – review & editing, Writing – original draft, Conceptualization. GU: Formal analysis, Methodology, Writing – review & editing, Data curation, Writing – original draft. MA: Writing – review & editing, Funding acquisition, Supervision, Writing – original draft, Software. FA: Writing – review & editing, Methodology, Formal analysis, Writing – original draft, Resources. RI: Data curation, Formal analysis, Writing – original draft, Resources, Writing – review & editing. ZE: Writing – original draft, Formal analysis, Writing – review & editing, Investigation, Validation. KJ: Supervision, Conceptualization, Writing – review & editing, Validation, Writing – original draft. LP: Writing – original draft, Writing – review & editing, Software, Data curation. KM: Writing – original draft, Writing – review & editing, Data curation, Resources. OS: Conceptualization, Project administration, Writing – review & editing, Methodology, Investigation, Writing – original draft.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that Generative AI was not used in the creation of this manuscript.

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