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Bioorganic fertilizers from agricultural waste enhance rice growth under saline soil conditions

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Agricultural waste (AW) presents significant environmental challenges if not effectively managed. Recycling AW as bio-organic fertilizers (BIOs) offers a sustainable solution, improving soil health, reducing dependence on chemical fertilizers, and stimulating crop growth. This study investigated the effectiveness of BIOs generated from AW composted with plant growth-promoting rhizobacteria (PGPR), including *Enterobacter* sp. R24, *Bacillus tequilensis* P8, and *Pseudomonas azotoformans* S81. BIOs produced from peanut shell, rice straw, duckweed, and rice bran were applied to rice seedlings under normal and saline (85 mM NaCl) conditions. The results revealed that PGPR-fermented BIOs utilized for only 15–30 days significantly improved seed germination and root length. BIO-duckweed and BIO-peanut proved high in nitrogen, phosphate, and potassium content, thereby increasing total biomass by 188% and 85%, respectively. In non-saline soil, BIO-peanut shell outperformed chemical fertilizers, promoting root growth and chlorophyll content. Additionally, BIO-rice straw gave a 58% reduction in proline levels under saline conditions, indicating stress reduction capacity. BIOs treatments demonstrated significant improvements in both nutrient availability and microbial diversity. Specifically, BIO-peanut shell and BIO-duckweed increased phosphate availability in soil by 143.26%, 13.80% over control soil and 7.23%, 30.69% over chemical treatment, respectively. The denaturing gradient gel electrophoresis (DGGE) analysis further revealed a noticeable increase in microbial diversity in soils treated with BIOs, which was absent in untreated soil. Indeed, BIO-rice straw promoted the development of five distinct bacterial genera in saline condition, underscoring BIOs' ability to enhance the microbial community structure. The study highlights the potential of BIOs from AW combined with PGPRs to enhance rice growth under extreme salt stress. This sustainable alternative to chemical fertilizers enhances soil health by increasing nutrient availability, microbial diversity, and promoting beneficial soil microbes, ultimately improving long-term soil resilience and fertility.

Keywords Agricultural waste, Bio-organic fertilizer, BIOs, Plant growth-promoting rhizobacteria (PGPR)

Abbreviations

AW	Agricultural waste
BIOs	Bio-organic fertilizers
BIO-duckweed	Bio-organic fertilizers made from duckweed
BIO-peanut shell	Bio-organic fertilizers made from peanut shell
BIO-rice bran	Bio-organic fertilizers made from rice bran
BIO-rice straw	Bio-organic fertilizers made from rice straw
DGGE	Denaturing gradient gel electrophoresis
EC	Electrical conductivity
FDA	Fluorescein diacetate
LSD	The least significant difference method
N	Nitrogen
OM content	Organic matter content
P	Phosphorus
PGPR	Plant growth promoting rhizobacterial
K	Potassium

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TSB Tryptic soy liquid medium

Rice (*Oryza sativa* L.) is a critical food source, providing sustenance for over half of the global population. Its importance extends beyond food security, contributing significantly to the economies of many countries. Despite its ability to thrive in waterlogged conditions, rice is highly sensitive to salinity, with a threshold electrical conductivity (EC) of 3 dS m^{-1}[1]. This salt sensitivity poses a major challenge to rice production, especially as salinity becomes a more prevalent issue due to climate change and poor agricultural practices. In addition to salinity, rice and other crops face a range of abiotic stresses that threaten yields and overall food security[2]. As the world's population grows, agriculture is under more pressure to fulfill rising food demand. Improving the stress tolerance of crops such as rice is critical to guarantee future food security. However, the prolonged and excessive application of chemical fertilizers to achieve ever-higher production targets has prompted concerns about detrimental effects on soil quality, water pollution, and human health[3]. Over time, these fertilizers may damage soil structure, accumulate dangerous chemicals in water sources, and endanger human health by contaminating water and food[4]. Thus, sustainable options that lessen reliance on chemical inputs are critical for ensuring long-term agricultural output.

One potential solution is the use of agricultural waste (AW) with plant growth-promoting rhizobacteria (PGPR) to create bio-organic fertilizers (BIOs)[5]. In recent years, the amount of AW generated globally has expanded dramatically, with most of the waste mismanaged owing to a lack of suitable management procedures[6]. This mishandling causes environmental issues, emphasizing the importance of appropriate repurposing solutions for AW. If not adequately handled, these large quantities of solid waste pose a danger to the environment and food security[7]. As a result, there is an increasing interest in converting AW into useful resources such as organic fertilizers, cultivation materials, and soil additives. BIOs, which are produced by inoculating PGPR with agro-industrial waste, are emerging as a viable way to increase soil health while lowering chemical inputs. They increase microbial diversity, creating a more robust ecosystem that enhances nutrient cycling and soil structure[8]. BIOs make important minerals such as phosphorus and potassium more available to plants, resulting in increased growth and more abundant harvests. Furthermore, the organic matter in BIOs strengthens soil structure by encouraging aggregation, which promotes aeration, drainage, and water retention. BIOs also promote beneficial microbial activity, inhibit soil-borne illnesses, buffer soil pH, and aid carbon sequestration, making them a viable alternative to chemical fertilizers in sustainable agriculture[9].

By recycling AW, agribusinesses follow circular economy concepts by producing sustainable energy and bio-based products from biomass feedstock[10]. AW materials including peanut shells, rice straw, duckweed, and rice bran are high in cellulose, hemicellulose, and lignin, making them great options for organic fertilizer production[11]. Previous studies found that composted AW increases soil carbon content, improves nitrogen cycling, and boosts plant development[12]. Specific AW materials have been shown to yield unique advantages. Peanut shells demonstrated the ability to aid seedling development in salty soils, duckweed provides a long-term supply of nitrogen and phosphate, and rice straw increases soil organic carbon and nutrient recycling[13]. For example, Sharm et al.[14] noted that incorporating nitrogen and rice straw affects nitrogen use efficiency, soil nitrogen pools and enzyme activity in rice-wheat. Enhancing the quality and efficiency of AW compost can be accomplished by adding vital nutrients, bioactive substances, or microorganisms. Prior studies demonstrated that the addition of PGPR to AW compost makes it biologically active and effective for seed germination and plant growth, soil rehabilitation and disease suppression[15,16]. Bibi et al.[17] reported that using black gram husks and peanut shell compost with PGPR as BIOs positively affects maize germination parameters and growth.

In addition, PGPR colonies form in plant roots, interacting with the plant, and operating as a biofertilizer by modifying the soil to be more favorable for plant growth[18]. Moreover, they produce certain substances and enzymes that mitigate the high oxidative stress under drought and salt stress[19]. Abiotic stress causes a variety of changes in plant growth due to effects on the overall morphological, biochemical, and molecular mechanisms in plants[20]. PGPRs interact with plants to greatly increase the accumulation of proline, a crucial osmo-protectant, in plant tissues under abiotic stress conditions such as salinity and dehydration. Proline is essential for maintaining cell osmotic balance, safeguarding cellular structures, and stabilizing proteins and membranes against stress-induced damage[21]. PGPRs promote proline biosynthesis by activating the expression of proline synthesis-related genes, allowing plants to adapt to and endure adverse situations[22]. PGPRs, specifically *Bacillus* and *Pseudomonas* strains, can increase plant proline accumulation by altering stress-related signaling pathways, particularly the abscisic acid (ABA) pathway, which is essential for plant stress responses[23]. The increased proline content caused by PGPRs mitigates the negative effects of stress by maintaining turgor and cellular integrity and contributes to the scavenging of reactive oxygen species (ROS), thereby lowering oxidative stress[24]. Studies have revealed that PGPR, such as *Bacillus* and *Enterobacter* species, can withstand salt and drought stress, while *Pseudomonas* species are an excellent alternative to chemical fertilization under saline circumstances[25]. Furthermore, PGPR accelerates the breakdown and nutrient release of AW compost, making it more biologically active and thus useful for seed germination, plant development, and soil repair. While previous study has proven the individual advantages of AW and PGPR, the combined impacts of various BIO formulations on soil microbial composition, crop production, biomass, and nutrient absorption in rice grown under saline conditions have yet to be investigated[26]. However, Yu et al.[27] reported that combining organic fertilizers, such as spent mushroom substrate compost, with beneficial microbes promoted pepper plant growth and suppressed plant disease by maintaining soil fertility.

This study aimed to create a novel BIO that reduces reliance on chemical fertilizers while also addressing environmental concerns about AW management. The BIOs were designed to improve soil organic matter, enhance physical and chemical soil properties, and boost enzyme activity and microbial diversity, all of which contribute to long-term soil sustainability. The strategy entailed combining PGPR with AW materials like peanut shells, rice straw, duckweed, and rice bran to produce biologically active BIOs. PGPRs were chosen for

their capacity to improve nutrient availability, root growth, and stress tolerance in rice under saline conditions by increasing proline buildup and antioxidant activity. This method not only increases plant growth and soil health but also promotes sustainable agriculture concepts by lowering chemical inputs and mitigating the environmental impact of AW.

Results

Increased microbial proliferation and survival in BIOs after one month of assessment

The microbial growth and survival of a co-inoculum containing three PGPR strains- *Enterobacter* sp. R24, *Bacillus tequilensis* P8, and *Pseudomonas azotoformans* S81 strains at a concentration of 10^8 CFU/ml in four distinct AW composition are illustrated in (Fig. 1). The objective was to determine the adaptation and resilience of these PGPR strains in different AW composts. Following the initial inoculation, all BIO formulations exhibited a significant rise in bacterial populations, peaking on day 7. Despite successive declines in microbial counts, log CFU ml^{-1} remained consistently between 10.89 and 11.25. This finding suggests that specific developmental stages or microbial life cycles are accomplished occur within BIOs formulations, emphasizing the variable responses of PGPR strains to distinct AW sources.

Characterization of the physical and chemical properties of BIOs after one month of decomposition by co-inoculating three PGPR strains

The physical and chemical features of biofertilizers produced from AW sources (peanut shell, rice straw, duckweed, and rice bran) and co-inoculated with three kinds of PGPR strains (*Enterobacter* sp. R24, *Bacillus tequilensis* P8, and *Pseudomonas azotoformans* S81) are presented in (Table 1). Over 30 days, the BIO-rice straw consistently maintained the highest moisture content, ranging from 53 to 61%. Incorporating BIO-duckweed led to a significant increase in moisture content, from 37% on day 0 to 48% on day 15, while BIO-peanut shell kept moisture levels between 35 and 44%.

All BIOs formulations maintained a consistent neutral pH throughout the trial, maintaining soil pH compatibility with no acidity or alkalinity variations. In terms of EC, BIO-duckweed exhibited the greatest variation, ranging from 6.4 to 7.5 dS m^{-1}. In contrast, BIO-rice straw, BIO-peanut shell, and BIO-rice bran had lower EC values, ranging from 2.0 to 3.8 dS m^{-1}. BIO-duckweed possessed high EC due to its aquatic origin and nutrient-dense composition, which allows it to absorb soluble salts and release minerals such as potassium, calcium, and magnesium[28]. These findings suggest that different AW sources, when utilized to make fertilizer and co-inoculated with PGPR strains, have distinct physical and chemical properties. BIO-rice straw consistently had the greatest moisture content, while BIO-duckweed showed considerable changes in both moisture content and EC values due to its nutrient-rich aquatic nature. The capacity of these BIOs formula to sustain neutral pH levels reinforces their viability for agricultural application.

Table 2 highlights variations in organic matter (OM), total nitrogen (N), phosphate (P), and potassium (K) content after 30 days of self-fermentation among four distinct BIOs. BIO-peanut shell (F1) had the highest OM content at 66.28%, followed by BIO-rice straw (F2) at 60.09%. BIO-duckweed (F3) exhibited the highest total N content at 2.54%, emphasising its potential as a nitrogen-rich biofertilizer, while F1 had the lowest nitrogen content at 1.23%. Additionally, F3 was found to have the greatest phosphate and potassium content at 0.46% and 3.74%, respectively, indicating its efficacy as a phosphorus and potassium-enriched biofertilizer. These

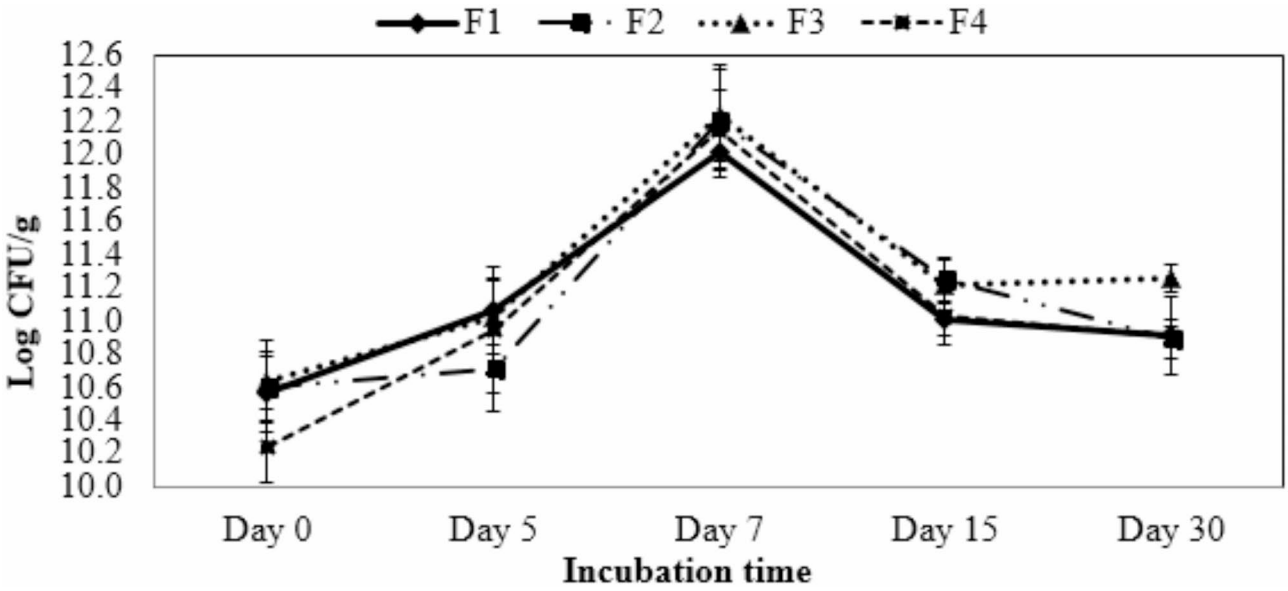

Fig. 1. PGPR population (log cfu/ml) survival ability after 30 days in four different agricultural waste components (F1: BIO-peanut shells, F2: BIO-rice straw, F3: BIO-duckweed, F4: BIO-rice bran).

Fermentation time	Formula	Moisture content (%)	pH	Electrical conductivity (dS m^{-1})
0 day	F1	43 ± 1.8^{b}	6.6 ± 0.1^{a}	3.8 ± 0.1^{b}
	F2	53 ± 2.3^{a}	6.4 ± 0.1^{b}	3.4 ± 0.5^{b}
	F3	37 ± 2.4^{b}	6.1 ± 0.1^{c}	7.5 ± 0.2^{a}
	F4	39 ± 5.2^{b}	5.8 ± 0.0^{d}	2.3 ± 0.2^{c}
	F-test	**	**	**
15 days	F1	44 ± 1.7^{b}	6.5 ± 0.1	2.7 ± 0.7^{b}
	F2	61 ± 2.9^{a}	6.6 ± 0.0	2.9 ± 0.7^{b}
	F3	48 ± 7.5^{b}	6.2 ± 0.1	6.9 ± 0.4^{a}
	F4	46 ± 1.0^{b}	6.4 ± 0.5	3.8 ± 0.4^{b}
	F-test	**	ns	**
30 days	F1	35 ± 2.0^{b}	6.8 ± 0.0^{a}	2.0 ± 0.3^{c}
	F2	57 ± 3.0^{a}	6.6 ± 0.0^{ab}	2.9 ± 0.4^{b}
	F3	42 ± 0.6^{b}	6.3 ± 0.0^{c}	6.4 ± 0.2^{a}
	F4	32 ± 4.0^{b}	6.4 ± 0.2^{bc}	3.3 ± 0.3^{b}
	F-test	**	**	**

Table 1. Physical and chemical properties of BIOs over the incubation period of 30 days with PGPRs. [ns]The data non-significant difference. **Significant different at 95% ($p < 0.01$). Different letters in the column represent significant differences among treatments ($P < 0.05$) according to the HSD test. F1: BIO-peanut shells, F2: BIO-rice straw, F3: BIO-duckweed, F4: BIO-rice bran.

Formula	OM (%)	Total *N* (%)	*P* (%)	K (%)
F1: BIO-peanut shells	66.28 ± 0.65^{a}	1.23 ± 0.00^{c}	0.11 ± 0.00^{d}	0.80 ± 0.00^{d}
F2: BIO-rice straw	60.09 ± 0.09^{b}	1.20 ± 0.00^{c}	0.16 ± 0.00^{c}	1.55 ± 0.00^{b}
F3: BIO-duckweed	50.48 ± 0.06^{d}	2.54 ± 0.02^{a}	0.46 ± 0.00^{b}	3.74 ± 0.04^{a}
F4: BIO-rice bran	54.04 ± 0.30^{c}	2.11 ± 0.00^{b}	1.31 ± 0.00^{a}	1.37 ± 0.00^{c}

Table 2. Organic matter, total nitrogen, phosphate, and potassium content after 30 days of self-fermentation in four distinct BIOs. Different letters in the column represent significant differences among treatments ($P < 0.05$) according to the HSD test.

differences highlight the distinct nutritional profiles of each BIO, implying that formulations may be customized to better match varying soil and crop requirements, increasing their value in sustainable agriculture operations.

Application of BIOs on rice seedling stage in pot expertiment

Impact of four BIO formulations on the rice seed germination index

We investigated the effect of four distinct BIOs formulations, fermented for varying lengths of time (0 days, 15 days, and 30 days) following a 7-day growth phase, on rice seedlings (Fig. 2). Seed germination rates varied from 80% to 100% across all treatments, indicating a strong germination impact even at the initial stages of BIOs synthesis (Fig. 3A). The seed germination index (GI) data suggested that BIO-peanut shell had the greatest influence on GI and root elongation after 15 days of fermentation, while another formulation outperformed the others after 30 days of fermentation (Fig. 3B, C). However, BIO-rice bran consistently showed weaker performance in promoting root elongation and germination index over the 30-day period. These findings imply that BIOs should be fermented for 15–30 days to efficiently enhance rice development, with BIOs generated from peanut shells and duckweed being particularly beneficial. These differences underline the need to choose optimal BIO formulations based on unique soil and crop requirements in order to enhance agricultural output and sustainability.

Impact on rice growth development

In a 30-day trial with rice plants in normal and saline soil (85 mM NaCl) (Fig. 4), the growth-promotion performance of BIOs was assessed through changes in plant height, root length, and biomass (Table 3). In normal soil, the chemical fertilizer treatment (T6) resulted in the most statistically significant plant height (91.3 cm). However, BIO-peanut shell (T2) boosted both plant height (73 cm) and root length considerably (32.6 cm), demonstrating its ability to promote root development within 30 days. In saline soil, no significant variations in root length were observed between the BIOs and chemical fertilizer treatments. Notably, the use of BIO-peanut shell and BIO-duckweed resulted in a 24% increase in shoot length compared to the control. These findings highlight the capacity of BIOs to help rice plants tolerate salt stress as a viable alternative to conventional fertilizers.

In terms of total biomass, the chemical treatment had the highest value, with a mean dry weight of 7.71 g dry, followed by BIO-peanut shell, with a mean dry weight of 5.89 g across both soil conditions (Table 3). All four

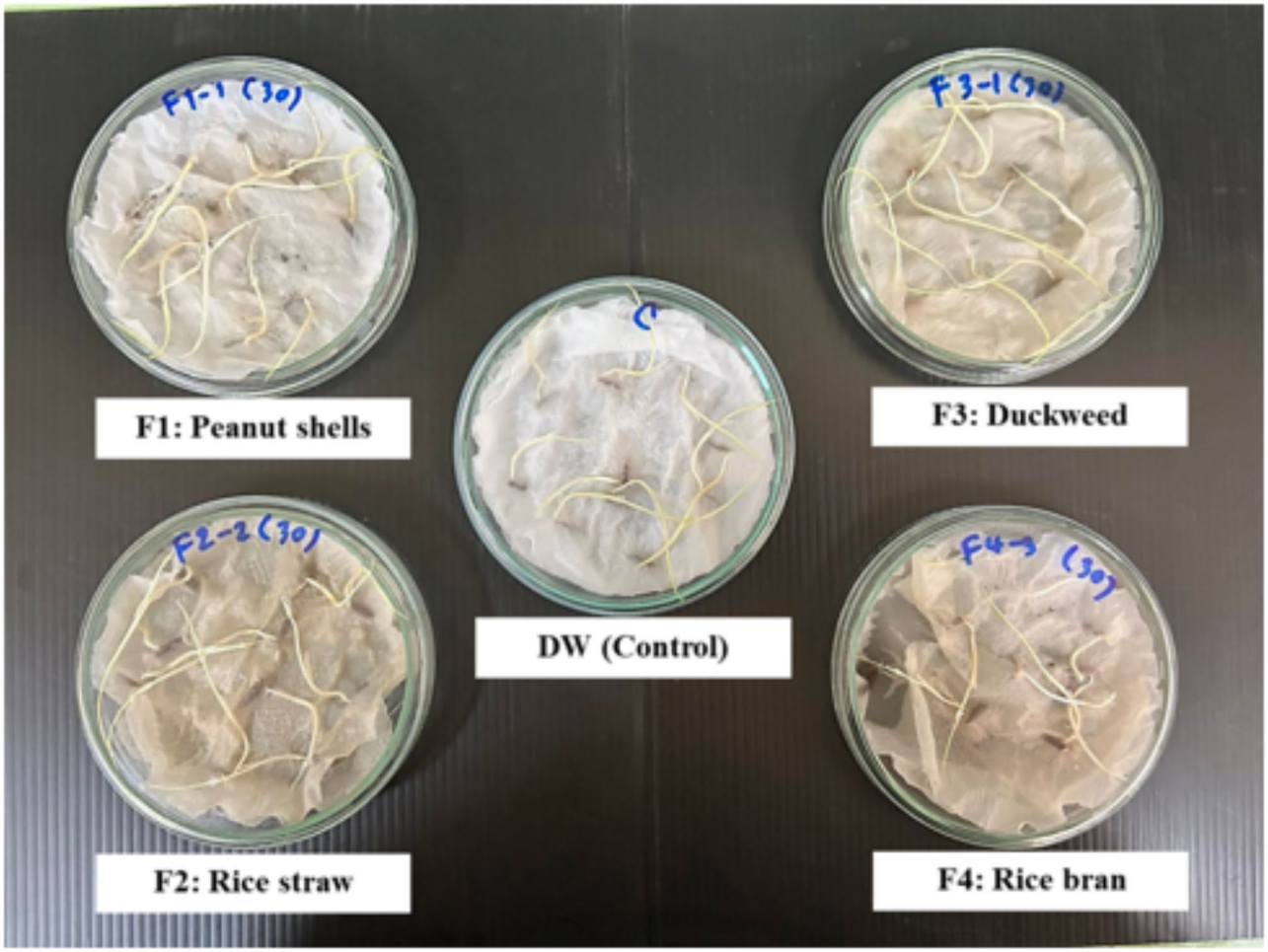

Fig. 2. Impact of four BIOs formulations (Day 30) on germination index values. The control treatment using distilled water (DW) is also included for comparison. Noted that F1: BIO-peanut shells, F2: BIO-rice straw, F3: BIO-duckweed, F4: BIO-rice bran.

BIOs formulations significantly raised biomass considerably compared to the control group that did not receive BIOs, demonstrating their capacity to promote plant development. Specifically, the BIO-peanut shell, BIO-rice straw, BIO-duckweed, and BIO-rice bran formulations increased total mean biomass by 188%, 45%, 85%, and 66%, respectively.

Chlorophyll concentration is an important measure of plant health; it varied significantly between treatments under both soil conditions in this research. In normal settings, BIO-peanut shell (T2), BIO-duckweed (T4), BIO-rice bran (T5), and chemical fertilizer (T6) had greater total chlorophyll levels than the control (T1). Treatment T2 exhibited the highest concentration (1.19 ± 0.22 mg g^{-1} FW), aligning with trends in chlorophyll a and b. Under saline soil conditions, BIO-duckweed (T10) showed the greatest total chlorophyll content (1.54 ± 0.40 mg g^{-1} FW). The present data demonstrate that BIOs treatments, particularly those derived from peanut shells and duckweed, effectively promote rice growth and resilience under both normal and saline conditions.

Effect of BIOs on Fluorescein Diaceatate (FDA) Hydrolysis and proline content under normal and salt stress

Under typical soil conditions, the BIO-rice bran treatment (T5) yielded the greatest FDA hydrolysis content (6.3 µg/mL) (Fig. 5). Both BIO-peanut shell and BIO-rice straw formulations outperformed the chemical fertilizer treatment (T6) in terms of FDA content, demonstrating the effectiveness of our biofertilizer formulations in making nutrients available to plants. However, salt stress caused a considerable drop in FDA content for both the BIO-peanut shell and BIO-rice straw treatments, showing that enzyme activity is limited under saline conditions. In contrast, the BIO-rice bran maintained a higher FDA concentration, indicating that this treatment was more robust and better sustained microbial populations. .

In both soil conditions, all BIOs had considerably lower proline levels than the chemical fertilizer treatment or control (Fig. 5). The BIO-rice straw treatment had a proline level of 20.5 µg/ml, which is 58% lower than the control in saline conditions, indicating that rice straw biofertilizer might help mitigate salt stress. The observed increase in proline concentration in salt-stressed rice plants may be due to an adaptive response to salt stress[22]. The use of BIOs treatment mitigated the severity of salt stress in rice and has the potential to retain enzyme activity and the stability of microbial populations, hence improving plant health.

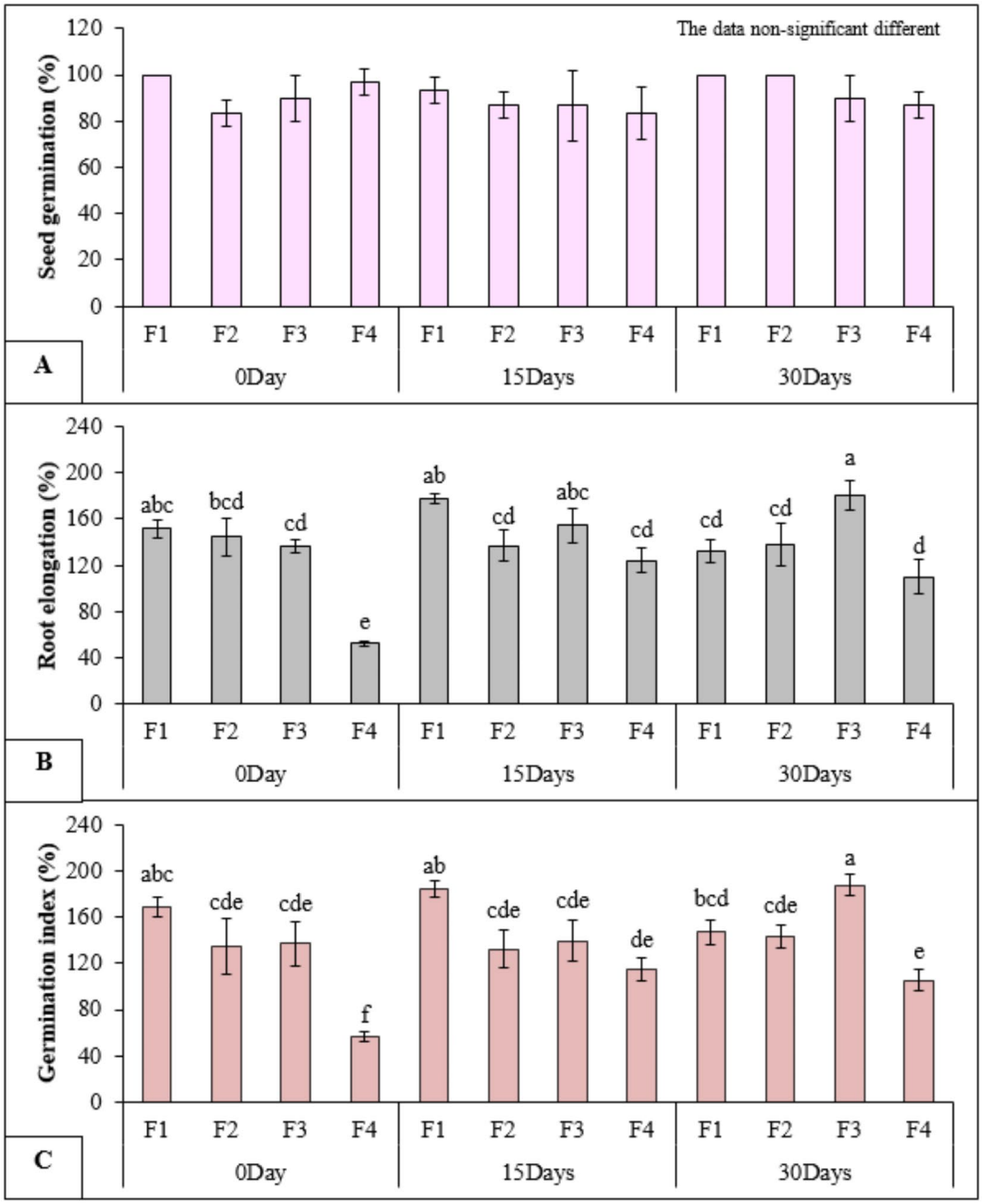

Fig. 3. Impact of four different BIOs formulations on rice seedling growth across various fermentation periods (0 days, 15 days, 30 days). Different letters in the column represent significant differences among treatments ($P < 0.05$) according to the HSD test. Noted that F1: BIO-peanut shells, F2: BIO-rice straw, F3: BIO-duckweed, F4: BIO-rice bran.

Change in soil properties and soil microbial community affected by BIOS

This study examined the effects of applying BIOs on soil properties over a 30-day period. The results revealed that using BIOs considerably enhanced organic content, available phosphorus, and exchangeable potassium compared to the control and chemical fertilizer. Under normal soil conditions, the application of various BIOs resulted in significant increases in OM content: 205.56% for BIO-peanut shell, 115.56% for BIO-rice straw, 51.11% for BIO-duckweed, and 103.33% for BIO-rice bran formulations. These data show that BIOs, particularly those derived from peanut shell, rice straw, and rice bran, were much more effective than chemical fertilizers in raising OM content.

Similarly, in saline soil conditions, BIOs dramatically enhanced OM content, with the BIO-peanut shell formula increasing organic matter by 43.75% compared to chemical treatment. Unexpectedly, there was no discernible difference in total nitrogen levels between normal and saline conditions. However, Fig. 6 reveals a considerable increase in exchangeable potassium (K) values with the addition of BIO-peanut shell in normal soil

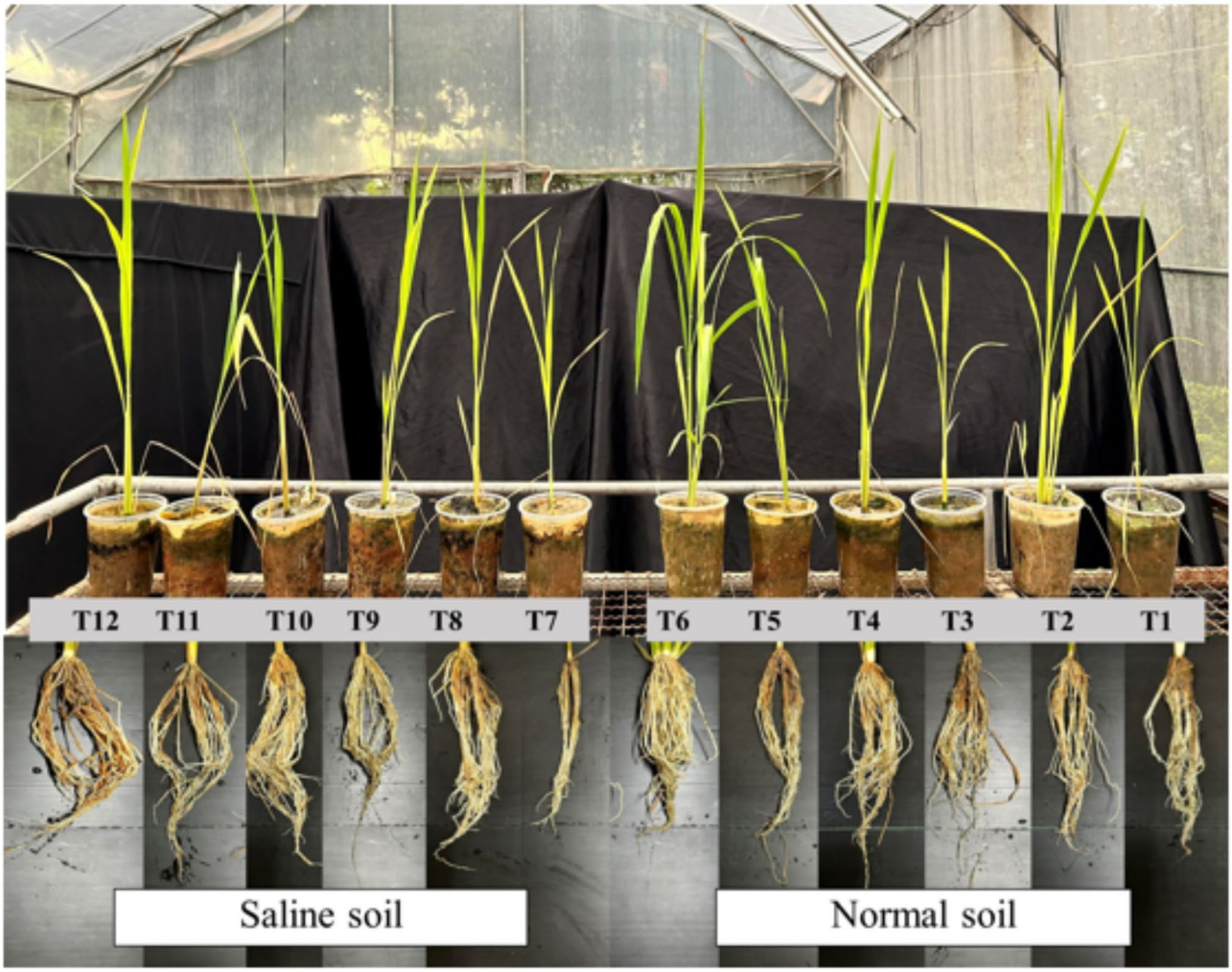

Fig. 4. A photograph of rice plants and rice roots after 30 days of planting. Note that T1: Control in normal soil, T2: BIO-peanut shell treatment in normal soil, T3: BIO-rice straw treatment in normal soil, T4: BIO-duckweed treatment in normal soil, T5: BIO-rice bran treatment in normal soil, T6: Chemical fertilizer in normal soil, T7: Control in saline soil, T8: BIO-peanut shell treatment in saline soil, T9: BIO-rice straw treatment in saline soil, T10: BIO-duckweed treatment in saline soil, T11: BIO-rice bran treatment in saline soil, T12: Chemical fertilizer in saline soil.

Condition	Treatments	RL (cm)	SL (cm)	SDW (g)	RDW (g)	Biomass (g)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Chl (a + b) (mg g^{-1} FW)
Normal soil	T1: Control	22.3 ± 1.5^{ab}	61.0 ± 1.0^{bcde}	0.3 ± 0.08^{d}	0.6 ± 0.16^{efg}	0.9 ± 0.24^{ef}	0.48 ± 0.06^{de}	0.74 ± 0.09^{ab}	0.74 ± 0.09^{b}
	T2: BIO-peanut shells	32.6 ± 5.5^{a}	73.0 ± 7.0^{ab}	1.4 ± 0.24^{b}	1.9 ± 0.3^{ab}	3.4 ± 0.77^{b}	1.02 ± 0.18^{ab}	0.05 ± 0.05^{b}	1.19 ± 0.22^{ab}
	T3: BIO-rice straw	23.6 ± 4.0^{ab}	44.6 ± 4.5^{e}	0.3 ± 0.05^{d}	$1.6 \pm 0.18^{a-d}$	1.6 ± 0.24^{cde}	0.43 ± 0.04^{de}	0.07 ± 0.05^{ab}	0.65 ± 0.19^{b}
	T4: BIO-duckweed	26.6 ± 4.6^{ab}	68.0 ± 1.7^{bc}	0.7 ± 0.09^{cd}	$0.9 \pm 0.19^{c-g}$	1.7 ± 0.28^{cde}	0.77 ± 0.10^{bcd}	0.06 ± 0.04^{ab}	0.97 ± 0.21^{ab}
	T5: BIO-rice bran	25.6 ± 4.1^{ab}	$58.6 \pm 4.5^{b-e}$	0.6 ± 0.11^{cd}	$1.6 \pm 0.21^{a-d}$	2.3 ± 0.32^{cd}	0.83 ± 0.12^{bc}	0.07 ± 0.01^{ab}	1.07 ± 0.08^{ab}
	T6: Chemical fertilizer	23.0 ± 1.0^{ab}	91.3 ± 3.0^{a}	2.7 ± 0.45^{a}	2.3 ± 0.17^{a}	5.0 ± 0.63^{a}	0.75 ± 0.12^{bcd}	0.08 ± 0.01^{ab}	1.00 ± 0.17^{ab}
Saline soil	T1: Control	25.6 ± 3.7^{ab}	48.3 ± 6.9^{de}	0.4 ± 0.07^{d}	1.7 ± 0.10^{abc}	1.0 ± 0.17^{e}	0.65 ± 0.06^{cde}	0.05 ± 0.02^{ab}	0.83 ± 0.14^{ab}
	T2: BIO-peanut shells	26.3 ± 3.5^{ab}	$60.0 \pm 5.5^{b-e}$	0.5 ± 0.10^{cd}	$0.5 \pm 0.26^{a-f}$	2.4 ± 0.32^{cd}	0.60 ± 0.07^{cde}	0.08 ± 0.03^{ab}	0.85 ± 0.16^{ab}
	T3: BIO-rice straw	26.0 ± 5.1^{ab}	50.0 ± 5.7^{cde}	0.5 ± 0.14^{cd}	0.7 ± 0.12^{efg}	1.2 ± 0.27^{de}	0.33 ± 0.02^{e}	0.03 ± 0.00^{b}	0.45 ± 0.02^{b}
	T4: BIO-duckweed	26.0 ± 2.0^{ab}	$60.3 \pm 4.0^{b-e}$	0.6 ± 0.07^{cd}	$1.5 \pm 0.31^{a-d}$	2.0 ± 0.35^{c}	1.19 ± 0.23^{a}	0.11 ± 0.06^{a}	1.54 ± 0.40^{a}
	T5: BIO-rice bran	18.3 ± 2.0^{b}	47.6 ± 3.2^{de}	0.2 ± 0.06^{d}	$0.8 \pm 0.19^{d-g}$	1.0 ± 0.26^{e}	0.74 ± 0.09^{bcd}	0.09 ± 0.00^{ab}	1.01 ± 0.12^{ab}
	T6: Chemical fertilizer	26.3 ± 3.5^{ab}	63.6 ± 4.7^{bcd}	1.0 ± 0.11^{bc}	$1.6 \pm 0.33^{a-d}$	2.6 ± 0.32^{bc}	0.49 ± 0.11^{cde}	0.05 ± 0.01^{ab}	0.66 ± 0.16^{b}

Table 3. Effect of agricultural waste biofertilizer - BIOs on different growth parameters of rice plant after 30 days planting. Data are represented as average of five replicates. *RL* root lengthm, *SL* shoot length, *SDW* root dry weight, *SFW* shoot dry weight. Different letters in the column represent significant differences among treatments ($P < 0.05$) according to the HSD test.

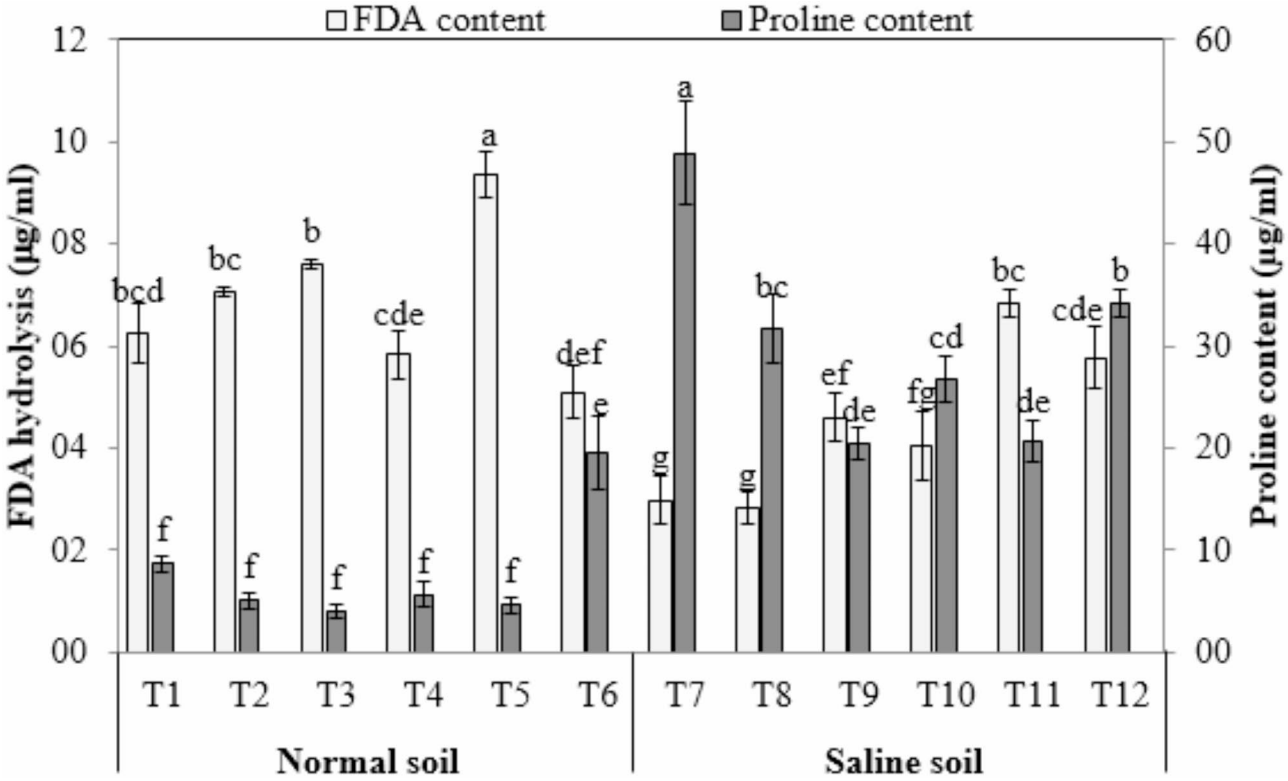

Fig. 5. The Fluorescein diaceatate hydrolysis and proline content of rice plant under 2 kinds of soil conditions. Small alphebetic letters indicat significant differences among the means of all treatment by HSD, $P \leq 0.05$. (T1: Control in normal soil, T2: BIO-peanut shell treatment in normal soil, T3: BIO-rice straw treatment in normal soil, T4: BIO-duckweed treatment in normal soil, T5: BIO-rice bran treatment in normal soil, T6: Chemical fertilizer in normal soil, T7: Control in saline soil, T8: BIO-peanut shell treatment in saline soil, T9: BIO-rice straw treatment in saline soil, T10: BIO-duckweed treatment in saline soil, T11: BIO-rice bran treatment in saline soil, T12: Chemical fertilizer in saline soil).

(94.74%) and BIO-duckweed in saline soil (13.08%) as compared to untreated soil. Furthermore, using BIO-peanut shell increased phosphate (P) availability by 143.26% compared to untreated soil and 7.23% compared to chemical treatment under standard conditions. Meanwhile, BIO-duckweed enhanced P availability by 13.80% compared to the control treatment and 30.69% compared to chemical treatment under standard conditions. These findings demonstrate the efficacy of BIOs in enhancing soil characteristics and nutrient availability, establishing them as a viable alternative to traditional chemical fertilizers for sustainable agriculture.

DGGE analysis of microorganism's communities in soil samples

Denaturing gradient gel electrophoresis (DGGE) is a powerful molecular technique for separating DNA fragments of equal lengths but different sequences. When applied to 16S rRNA genes, DGGE allows for a thorough phylogenetic investigation of microbial communities[29]. Each DGGE band typically represents at least one ribotype[30]. According to the study, BIOs had significant effects on the structure of the soil bacterial population, enhancing bacterial diversity. For example, around five bacterial genera developed in the T9 treatment (BIO-rice straw in saline soil) and the T12 treatment (chemical fertilizer in saline soil), while the BIO-rice bran formulation treatment exhibited around four bacterial groups in both situations (Fig. 7). This increase in diversity is most likely due to the direct influence of the inoculated microorganisms in the BIOs or their ability to promote the growth of indigenous soil microorganisms. In addition, distinct band patterns in the DGGE profiles from the BIOs treatment, which were lacking in the control, showed the development of new ribotypes. This demonstrates the potential of BIOs to increase microbial diversity in soil, which can be beneficial for rice cultivation.

Discussion

Soil salinization remains one of the most critical obstacles to agricultural production and food security, particularly in Southeast Asia. The Mekong Delta, a major rice-producing region, is becoming increasingly vulnerable to salinity intrusion, which reached record levels in 2020, extending over 110 km inland and damaging roughly half a million hectares of agricultural land[31]. By 2050, salinity is expected to affect up to half of the world's arable land[32]. Rising groundwater salinity, poor drainage and irrigation infrastructure, and excessive use of chemical fertilizers are among the key causes of soil salinization[33]. These difficulties necessitate the development of techniques to improve rice plant tolerance to salinity, ensuring crop survival and yield in

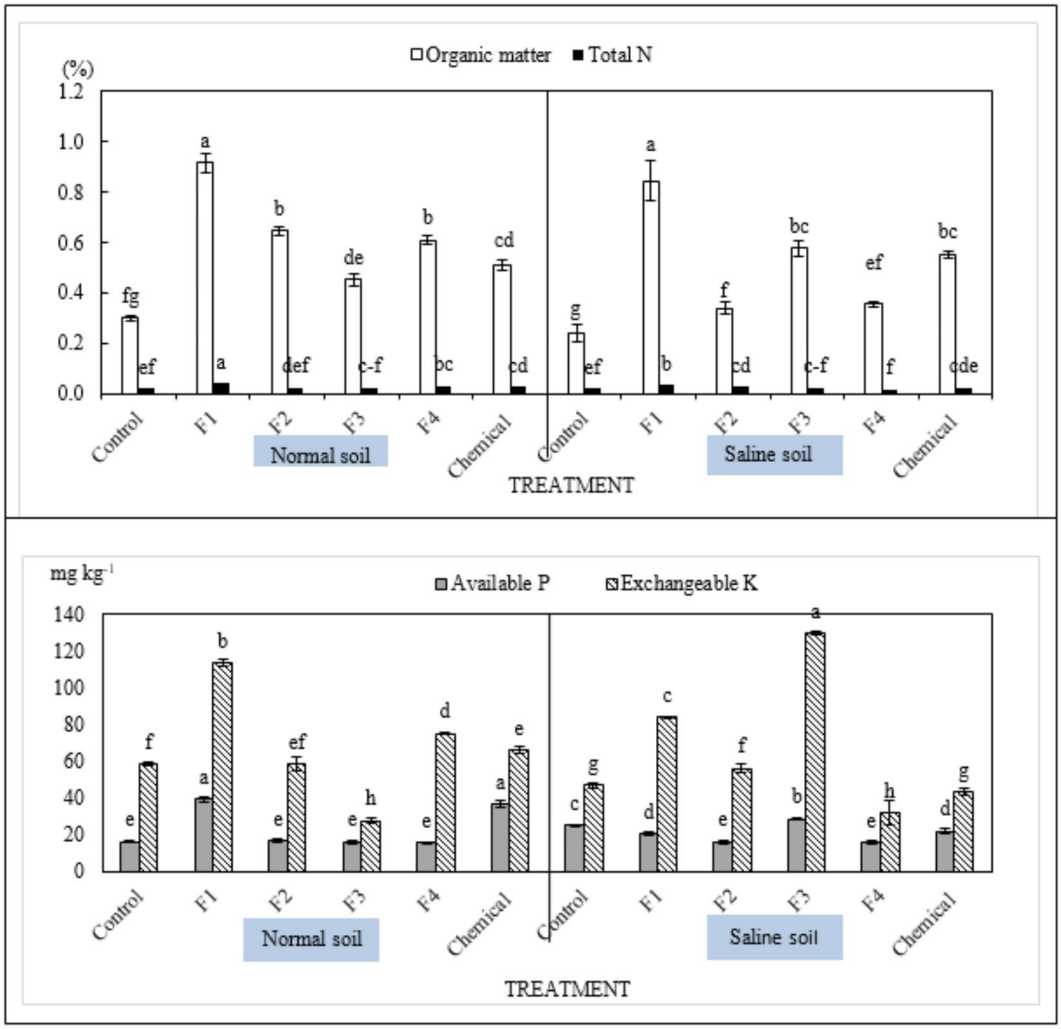

Fig. 6. The effect of BIOs from various agricultural waste material on organic matter content, total nitrogen, available phosphate, and exchangeable potassium in normal and saline soils after 30 days of rice plant cultivation. Small alphebetic letters indicat significant differences among the means of all treatment by HSD, $P \leq 0.05$. Noted that F1: BIO-peanut shells, F2: BIO-rice straw, F3: BIO-duckweed, F4: BIO-rice bran.

affected areas. One effective approach is to introduce organic amendments such as animal manure, compost, and cover crops, boost soil microbial activity by providing carbon sources that encourage organic matter decomposition and the release of vital nutrients[34]. This procedure not only promotes plant growth but also raises soil organic carbon levels, improving soil structure and water retention capacity[31]. Furthermore, halotolerant plant growth-promoting rhizobacteria (PGPR), especially when combined with organic amendments, might enhance plant resistance to salt stress[35]. The use of organic fertilizers, such as biofertilizers (BIOs), can lower salt levels by improving soil structure and nutrient cycling. The organic matter (OM) in BIOs acts as a nutrient reservoir, slowly releasing nutrients over time and reducing the likelihood of leaching and runoff[36]. This efficient nutrient management helps maintain a balanced soil ecosystem by preventing salt accumulation, which is a major contributor of soil salinity.

In this context, the current study investigated the efficacy of BIOs derived from diverse agricultural waste (AW) materials in promoting rice development and reducing soil salinity. The results showed that using specific BIOs, such as those generated from peanut shells, rice straw, and duckweed, greatly improved microbial proliferation, soil characteristics, and plant growth. Specifically, the BIOs supported the growth and survival of PGPR strains including *Enterobacter* sp. R24, *Bacillus tequilensis* P8, and *Pseudomonas azotoformans* S81, which showed a substantial increase in bacterial populations, peaking at day 7 and maintaining elevated CFU levels throughout the 30-day trial. These data imply that BIOs promote the proliferation and robustness of PGPR, resulting in improved plant growth under both normal and saline circumstances. Many studies have reported a significant increase in agricultural productivity with the co-application of AW and PGPR rather than the sole application of PGPR or AW materials[37]. BIO-rice straw maintained the highest moisture content, while BIO-duckweed showed a considerable increases in both moisture content and EC due to its nutrient-rich aquatic nature (Table 1). In addition, in terms of nutrient content, BIO-duckweed formula exhibited the highest levels of total nitrogen,

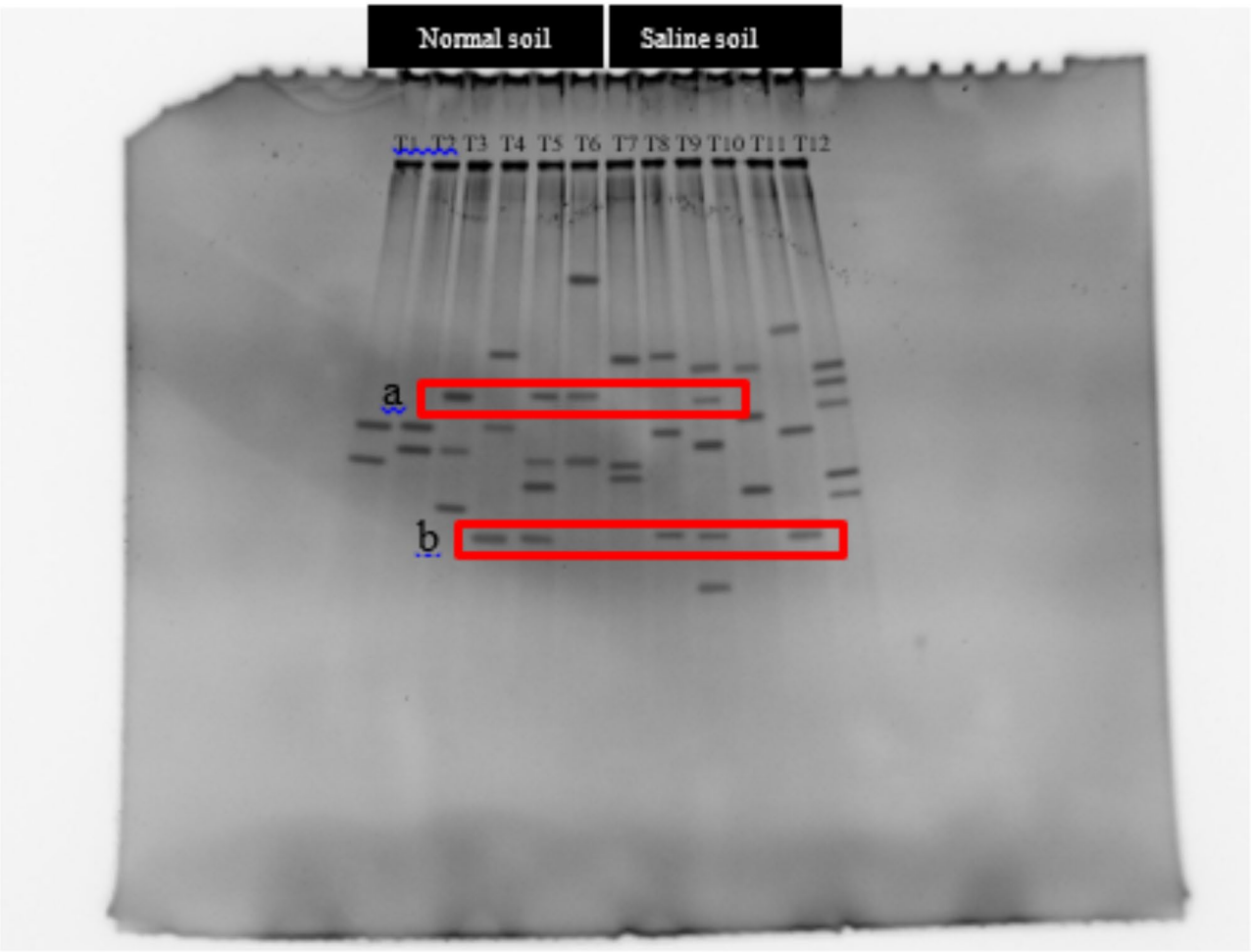

Fig. 7. DGGE banding patterns of bacterial 16 S rRNA gene contained in soil samples planting rice plant 30 days. Note that T1: Control in normal soil, T2: BIO-peanut shell treatment in normal soil, T3: BIO-rice straw treatment in normal soil, T4: BIO-duckweed treatment in normal soil, T5: BIO-rice bran treatment in normal soil, T6: Chemical fertilizer in normal soil, T7: Control in saline soil, T8: BIO-peanut shell treatment in saline soil, T9: BIO-rice straw treatment in saline soil, T10: BIO-duckweed treatment in saline soil, T11: BIO-rice bran treatment in saline soil, T12: Chemical fertilizer in saline soil.

phosphate, and potassium, making it an effective phosphorus and potassium-enriched biofertilizer (Table 2). The distinct nutritional profiles of each BIO formulation offer the potential for customization to address various soil fertility and crop needs, enhancing their value in agricultural applications.

Soil salinity has been shown to diminish rice output by around 29.29% compared to non-saline soils, with technical efficiency decreasing in saline soils. Rice cultivation with saline soil has an average technical efficiency of just 73%, whereas with non-saline soil that figure might approach 80%[38]. This emphasizes the necessity of devising measures that not only minimize the immediate consequences of salinity but also improve long-term soil health and microbial biodiversity. Zhang et al. discovered that the application of composted poultry manure as an organic fertilizer is an effective measure for remediating saline-alkali soil and enhancing rice growth[39]. Xiao et al. proposed that organic fertilizer mixed with soil amendments significantly improves soil quality, promoting rice growth and production by reducing soil salinity and increasing soil biochemical features in coastal saline soil[40]. In line with this, our study demonstrated that the application of BIOs led to significant improvements in seed germination, plant height, root length, and biomass. Moreover, the BIO-peanut shell and BIO-duckweed formulations showed notable increases in shoot length and biomass under both normal and saline soil conditions (Fig. 3). This indicates their potential to enhance rice growth and resilience, offering a viable alternative to traditional chemical fertilizers. Moreover, BIO-peanut shell and BIO-duckweed formulations were especially effective in enhancing soil properties, further supporting their role in sustainable agriculture. These formulations also enhanced soil organic matter content, available phosphorus, and exchangeable potassium, highlighting their importance in promoting soil health and fertility (Fig. 6).

The accumulation of proline, an amino acid that functions as an osmoprotectant, is an important physiological indicator of salinity-induced plant stress[41]. The reduction in proline levels seen after BIOs treatment can be explained by proline's dual role as an osmoprotectant and a stress signal (Fig. 5). Plants often store proline in

response to stress, such as salt stress, to protect cellular structures and maintain osmotic equilibrium[20]. However, the lower proline accumulation seen in BIO-treated plants shows that they endured less salt stress than the untreated control. This suggests that the BIOs were efficient at mitigating the effects of salt, reducing the need for excessive proline accumulation[32]. This study concludes that using BIOs to promote a healthier root environment, increase nutrient availability, and boost beneficial microbial activity likely helped plants manage high salinity more effectively, reducing the physiological stress response. Lower proline levels in treated plants are thus a positive signal, indicating reduced stress exposure and enhanced plant resilience, ultimately leading to better plant growth and production under saline circumstances.

Previous research has highlighted the effectiveness of co-applying organic materials like rice straw, PGPR, and other AW resources to enhance soil fertility and mitigate salinity effects[42]. Moreover, inoculation promotes the breakdown of complex chemical compounds, releasing nitrogen and other nutrients for plant absorption; the carbon-to-nitrogen ratio in rice straw stimulates microbial activity, which improves nutrient cycling and increases total nitrogen in fields[43].Ibrahim et al.[44] discovered that BIO-peanut shell improved nutrient mobilization and solubilization, resulting in the release of phosphorus from organic sources. Our research supports previous findings, highlighting BIOs' potential as an alternative to traditional chemical fertilizers. Furthermore, the use of AW materials improves nutrient cycling while also reducing nitrogen, phosphorus, and potassium leaching, resulting in increased soil fertility and lower environmental contamination.

Indeed, the utility of duckweed has emerged as a viable AW resource, with earlier studies revealing its role in enhanced nutrient absorption, higher chlorophyll content, and increased plant biomass in treated crops[45]. Duckweed is well-known for its ability to accumulate phosphorus and its high in protein and free nitrogen[46]. Fall et al.[13] reported that the use of peanut shell amendments improved plant growth, enhanced soil chemical properties, and reduced soil salinity by increasing total soil carbon, total N, P and FDA, regardless of salinity. Rice straw contains beneficial nutrients that aid in plant growth and maintain soil fertility. Liang et al.[47] demonstrated that a special compound fertilizer incorporated with rice straw increased fruit yield by altering soil carbon fractions. Adegoke et al.[48] found that rice bran and cow dung compost boosted soybean yields. While the benefits of BIOs are evident, some formulations, such as BIO-rice bran, did not exhibit the same level of efficacy in promoting plant development as others (Fig. 3). Such variation implies that the intricate interactions between organic amendments and PGPRs may affect biofertilizer effectiveness. Rice bran's rich organic makeup may aid in microbial hydrolysis, but certain compounds or allelopathic substances may impede plant development, emphasizing the need for further research into the optimal formulation and mix of BIOs[49].

The present study supports the hypothesis that using AW such as rice straw, duckweed, peanut shell for BIOs formulation effectively promotes rice plant growth and resilience under both normal and severely saline conditions. Indeed, the application of these BIOs formulations on rice seedlings demonstrated significant improvements in seed germination, plant height, root length, and biomass (Table 3). BIO-peanut shell and BIO-duckweed formulations showed notable increases in shoot length and biomass even under salinity stress. Moreover, BIOs treatments resulted in increased organic matter content, available phosphorus, and exchangeable potassium in the soil (Fig. 6). BIO-peanut shell and BIO-duckweed formulations were especially effective in enhancing these soil properties, further supporting their role in sustainable agriculture. The application of organic fertilizer improves leaf water content, nutrient uptake, nutrient homeostasis, synthesis of chlorophyll, osmolytes, hormones, secondary metabolites, antioxidant activities and gene expression, resulting in improved tolerance against drought, salinity, heat, and heavy metals[50]. The study also discovered increases in all photosynthetic pigments in rice plants under salinity stress conditions. An increase in chlorophyll content in PGPR-treated plants could be attributed to the formation of a number of microbial metabolites that arise during water and mineral absorption and aid in the facilitation of photosynthetic material in plant cells[51]. These results suggest that BIOs represent a viable and sustainable alternative to chemical fertilizers in rice cultivation, offering multiple benefits, including improved plant growth, enhanced soil fertility, and reduced environmental impact.

Several studies have demonstrated that mixing PGPR with organic substrates to create BIOs can boost PGPR activity[17,52,53]. Sritongon et al.[54] found that these PGPR strains boost rice growth under stress and improve nutrient availability in the rhizosphere. Ansari et al.[55] reported that *Pseudomonas azotoformans* effectively alleviated drought stress in wheat plants via a variety of biochemical processes. Liquid organic fertilizer mixed with *Enterobacter cloacae* enhanced P solubility, resulting in an increase in soil available P, reducing P fertilization by 50% and increasing maize production[56]. *Bacillus tequilensis* suppressed fungal *Fusarium solani* growth by releasing antifungal chemicals and proteins linked with plant stress resistance/tolerance, making it a viable candidate biocontrol agent against *Rehmannia glutinosa* root-rot disease[57]. However, the efficiency of PGPRs may vary depending on the climate and ecological zone. In field-growing conditions, some bacterial strains may struggle to colonize plant roots successfully. To mitigate such issues, crop types and salt-tolerant PGPR strains must be carefully chosen. It is also critical to find the optimal ratio and formula of PGPR and BIOs to promote plant growth in saline circumstances. This investigation confirmed that the addition of *Enterobacter* sp. R24, *Bacillus tequilensis* P8, and *Pseudomonas azotoformans* S81 increased nutrient solubility in AW. Furthermore, the utilization of indigenous PGPR strains as fertilizers may address concerns about bringing microbial inoculants into new environments.

The study of soil nutrients after 30 days of rice planting found that BIOs generated from peanut shells, rice straw, and rice bran were more effective than chemical fertilizers in boosting the organic matter (OM) content (Fig. 6). The integration of AW materials, as well as the reported improvements in rice growth and total plant biomass, likely contributed to this development. This growth may result from greater photosynthesis, as well as improved nutrient transport and accumulation[58]. The use of AW materials not only increased total organic carbon, total nitrogen, and total phosphorus levels in the soil, but also enhanced the availability of plant-accessible nutrients. Organic fertilizers reduced leaching losses of nitrogen, phosphorous, and potassium while increasing soil fertility retention capacity[59]. Moreover, microorganisms found in the BIOs performed a key role in transforming

otherwise inaccessible nutrient into plant-available forms, resulting in higher levels of available phosphorus (P) and exchangeable potassium (K) compared to untreated soil conditions[60]. This nutrient transformation and improvement might be critical in rehabilitating degraded soil, making BIOs viable alternative to conventional chemical fertilizers for sustainable agriculture. Denaturing Gradient Gel Electrophoresis (DGGE) is a useful approach for comparing bacterial community structures after soil treatment or under diverse land uses[61]. DGGE analysis indicated that adding BIOs boosted microbial diversity, which is good for soil health and plant growth (Fig. 7). The findings demonstrate that BIOs increase soil nutrient availability and foster a more diverse and resilient microbial ecology.

Bio-based fertilizers improve soil health and nutrient mineralization while offering long-term benefits to agribusinesses. Crucially, this study demonstrates the effectiveness of BIOs in reducing the negative consequences of salt stress. However, widespread adoption requires assessment of scalability, cost-effectiveness, and policy integration. It is worth noting that governments the world over are progressively supporting organic farming through subsidies and regulatory incentives, with frameworks such as those in the European Union identifying bio-based fertilizers (BBFs) as critical components of sustainable agriculture[62]. Bio-based fertilizers offer major advantages over conventional mineral fertilizers since they recycle nutrient-rich wastes while consuming less energy and nonrenewable resources[63]. However, issues such as heavy metal and pathogen contamination must be addressed by technical innovation and rigorous environmental assessment. Furthermore, bio-waste products can pollute the air due to odor emissions, particularly ammonia, if stored poorly. Additives used in composting can reduce emissions by increasing porosity and absorbing gases, resulting in higher product quality[64]. To stimulate widespread uptake of BIOs, streamlined regulatory frameworks, pilot trials, and public awareness activities are all required. Developing BIOs can be challenging but profitable, offering the ability to generate economic benefits. comprehensive approach that combines sustainable agriculture with ecological and social considerations is necessary. Future study should focus on economic viability, social acceptance, and obtaining policy backing to increase BIO production while formulating a comprehensive approach that combines sustainable agriculture with ecological and social considerations. Evaluating the techno-environmental performance of these fertilizers is critical for maximizing their long-term value. Ultimately, expanding BIOs research and development will unlock their full potential for increasing plant productivity and promoting environmental sustainability.

Conclusion

This study proved the potential benefits of bio-organic fertilizers (BIOs) in reducing salt stress in rice plants, improving crop growth and production. It introduced a novel way to producing BIOs from agricultural waste, providing a sustainable and long-term alternative to chemical fertilizers. The 30-day fermented BIOs, particularly those derived from rice straw, peanut shells, and duckweed, significantly improved soil fertility and rice development under salt stress. The BIO-duckweed and BIO-peanut shell formulations performed particularly well, resulting in considerable improvements in plant biomass, nutrient uptake, and stress tolerance. The observed decrease in proline levels demonstrated the efficacy of these BIOs to reduce plant stress. These findings present a route to sustainable agriculture by providing farmers with nutrient-rich, ecologically friendly solutions that improve soil health, boost crop productivity, and minimize pollution. The utilization of agricultural waste in the creation of BIOs promotes circular economy principles by providing a practical solution for recovering degraded soils and enhancing crop resilience. Nonetheless, more study into the scalability and flexibility of BIOs across various soil types, crops, and environmental circumstances is needed to fully realize their potential in sustainable agricultural systems. The long-term use of BIOs in rice cultivation shows potential for improving soil health, boosting nutrient availability, and increasing crop resilience. Future studies should examine the cumulative impacts of BIOs across numerous cropping cycles, specifically their impact on soil structure, organic matter content, and microbial diversity under continuous flooding circumstances. Furthermore, combining BIOs with other abiotic stress mitigation measures, such as employing salt- or drought-tolerant crop types, as well as biotic treatments, such as biological pest control or mycorrhizal fungi, may result in synergies. Field trials and multi-season research are critical for validating the practical viability, cost-effectiveness, and environmental impact of BIOs in real-world agricultural contexts. Furthermore, the performance of BIOs should be evaluated across different climatic zones and soil types to verify their efficacy and adaptability. By resolving these issues, BIOs have the potential to become a dependable option for increasing rice output under saline conditions, thereby contributing to agricultural sustainability and environmental protection.

Materials and methods

Collection of agricultural waste (AW) materials

Agricultural waste materials were collected from farms in Khon Kaen province, Thailand (the geographical coordinates are at 16.4837° N latitude and 102.7480° E longitude), including peat moss, peanut shells, rice straw, duckweed, and rice bran. All materials were dried at 100 °C for 48 h, ground into a fine powder, and sterilized at 121 °C for 1 h, repeated 2-3 times.

Plant growth promoting rhizobacterial (PGPR) inoculum

The PGPR strains (*Enterobacter* sp. R24, *Bacillus tequilensis* P8, and *Pseudomonas azotoformans* S81) were sourced from the Microbial Fertilizer Laboratory of the Department of Microbiology, Faculty of Science, Khon Kaen University[54]. PGPR strains were cultured in Trytic Soy Broth (TSB) at 30 °C for 24–48 h. The supernatant was collected by centrifugation at 4025 g, and the cell suspension was diluted with 0.85% NaCl, adjusted to a concentration of 10^8 CFU/ml for further experiments.

BIOs composition	F1 peanut shells	F2 rice straw	F3 duckweed	F4 rice bran
Peanut shell/ Rice straw/ Duckweed, Rice bran (g)	50	50	50	50
Peat moss (g)	30	30	30	30
Sodium alginate (g)	10	10	10	10
PGPRs (ml)	10	10	10	10
Water (ml)	40	40	40	40

Table 4. The compositions of BIOs formulation.

Agricultural waste bio-organic fertilizer (BIOs) production

The three PGPRs were mixed in a 1:1:1 ratio. BIOs compositions were prepared as outlined in (Table 4). The BIOs substrates were stored at room temperature, and microbial survival and humidity were monitored.

Characterization of BIOs

Using a pH meter and an EC meter, the pH and electrical conductivity (EC) of each BIOs formulation was evaluated in water extracts (BIOs: water ratio of 1:5 v/v). For moisture content, 10 g of sample BIOs was collected and distributed on a tray, then placed in a hot air oven at 105ºC for 24 h. The sample weight after drying was measured and computed according to the formula:

$$moisture\ content\ (\%) = \frac{Fresh\ weight\ -\ Dry\ weight}{Fresh\ weight} \times\ 100$$

The organic matter (OM) content in the BIOs was measured using the method outlined by Benton Jones[65]. Total nitrogen content was determined using the Kjeldahl method[66]. Available phosphorus was assessed via the ClO_4-H_2SO_4-molybdenum-antimony resistance colorimetric method[67].

Microbial survivial ability test for BIOs

To assess microbial survival, samples were obtained on days 0, 5, 7, 15, and 30. Approximately 10 g of BIOs samples were combined with 90 ml of distilled water and shaken at 2.52 g for 30 min. Serial dilutions were made, and the diluted samples were distributed onto plate count agar. After 24–48 h of incubation at 30 °C, total microbial counts were calculated and represented as log CFU ml^{-1}.

Effect of BIOs fermentation on seed germination and root elongation

Rice seedlings (RD6 Glutinous Rice) were provided by Salt-tolerant Rice Research Group, Faculty of Science, Khon Kaen University, Thailand (16°28'15.82"N, 102°49'11.48"E). All methods were performed in accordance with relevant guidelines and regulations of the Rice Department of Thailand. At specific intervals (day 0, day 15, and day 30), approximately 10 g of each BIOs formulation was mixed with 90 ml of distilled water, shaken at 2.52 g for 15–30 min, and filtered using No.1 filter paper to eliminate any residual substrates, yielding a filtered biofertilizer solution. Using a filtered biofertilizer solution during rice germination guarantees that seeds have access to beneficial microorganisms and dissolved nutrients without interference from leftover substrates. This avoids seed rot, fungal growth, and mechanical damage from solid particles, resulting in perfect conditions for uniform and healthy germination. For the germination test, ten rice seeds were uniformly placed on sterile petri dishes lined with sterilized tissue paper. The Ragdoll method, with appropriate modifications, was applied by sandwiching the seeds between two layers of sterilized tissue paper moistened with 3 mL of specific filtered biofertilizer solutions (F1, F2, F3, F4). For the control treatment, the seeds were moistened with 3 mL of distilled water. The Petri dishes were incubated in a dark environment for 5–7 days to promote germination under controlled conditions. The formula was as follows:

$$\text{Seed germination}\ (\%) = \frac{\text{Seed germination}}{\text{Total seed}}\ \text{X100}$$

$$\text{Root elongation} = \frac{\text{Root length of treatment}}{\text{Root length of control}}\ \text{x}\ 100$$

$$\text{Germination index}\ (\%) = \frac{\text{Seed germination}\ \text{x Root elongation of treatment}}{\text{Seed germination x Root elongation of control}}\ \text{x}\ 100$$

Effect of BIOs on rice growth in pot experiment

A pot experiment was conducted in a greenhouse under controlled settings, with temperatures ranging from 25 to 35 °C and daily irrigation. The objective was to assess the effect of bio-organic fertilizers (BIOs) on rice development in both normal and saline soils, with salinity generated by modifying the soil with 85 mM NaCl. The experiment used a randomized complete block design (RCBD) with five replications to ensure statistical rigor and reduce experimental error. The soil samples were collected from Khon Kaen province, Thailand (located on 16° 26′ N, 102° 50′ E), and contained 0.51% OM, 0.02% total N, 10.6 mg kg^{-1} available P and 25 mg kg^{-1} exchangeable K. Rice seedlings (RD6 Glutinous Rice) aged 15–20 days were transplanted into plastic pots

with a height of 30 cm and a diameter of 15 cm with each containing 1 kg of soil. Subsequently, 5 g of each BIOs formulation was applied according to the following treatments:

Normal soil (N) treatments:

1. Control pot in normal soil.
2. BIO-peanut shell formula (F1) (N).
3. BIO-rice straw formula (F2) (N).
4. BIO-duckweed formula (F3) (N).
5. BIO-rice bran formula (F4) (N).
6. Chemical fertilizer treatment (N).

Saline soil (S) treatments:

7. Control pot in saline soil.
8. BIO-peanut shell formula (F1) (S).
9. BIO-rice straw formula (F2) (S).
10. BIO-duckweed formula (F3) (S).
11. BIO-rice bran formula (F4) (S).
12. Chemical fertilizer treatment (S).

After one month of growth, the rice plants were harvested and various plant parameters were measured. Additionally, key soil properties, such as pH, EC were analyzed. To measure soil pH, 10 g of soil sample was mixed with 10 ml of distilled water (1:1). The mixture was allowed to stand for 5 min, after which the pH was determined using a pH meter. For EC analysis, 5 g of soil was mixed with 25 mL of distilled water in a 1:5 ratio. After thorough stirring, the EC was measured using an electrical conductivity meter. Soil enzyme activity was assessed using the fluorescein diacetate (FDA) method. One g of soil was mixed with 7.5 ml 60 mM sodium phosphate buffer (pH 7.6) and 0.1 ml fluorescein diacetate. The mixture was shaken at 150 rpm for 40 min. The reaction was then terminated by adding 7.5 mL of a chloroform: methanol solution (2:1). The resulting mixture was centrifuged at 8,000 rpm for 10 min. The absorbance of the upper layer of the solution was measured at 490 nm using a spectrophotometer, and FDA activity was quantified by comparing the absorbance values with a standard FDA calibration curve[68]. All methods were performed in accordance with relevant guidelines and regulations of the Rice Department of Thailand.

Proline content determination in rice leaves

Leaf proline content was determined following a modified procedure by Bates et al.[69]. A 0.5 g sample of leaves was extracted with 10 ml of 3% sulfosalicylic acid, filtered, and combined with 2 mL of the extracted solution, 2 mL of acid ninhydrin, and 2 mL of glacial acetic acid. The mixture was heated at 100 °C for an hour, and after cooling, toluene was added. The organic phase was measured using a spectrophotometer at 520 nm.

Chlorophyll content determination in rice leaves

Chlorophyll pigments were extracted using the method outlined by Palta[70]. Chlorophyll a and b levels in the samples were calculated using the following formula:

$$chlorophyll\ a\ \left(\frac{mg}{g}\right) = \frac{(12.7 \times\ \mathrm{A663} - 2.69 \times\ \mathrm{A645}) \times\ \mathrm{V}}{1000 \times\ \mathrm{W}}$$

$$chlorophyll\ b\ \left(\frac{mg}{g}\right) = \frac{(22.9 \times\ \mathrm{A645} - 4.86 \times\ \mathrm{A663}) \times\ \mathrm{V}}{1000 \times\ \mathrm{W}}$$

(Where: V = volume of the extraction solvent in each sample (ml), W = weight of fresh leaf (g))

Evaluation of soil nutrient availability

The organic matter (OM) content in the soil was measured using the method outlined by Benton Jones[65]. Total nitrogen content was determined using the Kjeldahl method[66]. Available phosphorus was assessed via the ClO_4-H_2SO_4-molybdenum-antimony resistance colorimetric method[67]. Exchangeable potassium was measured using NH_4OAc extraction by flame photometry[71].

Detection of microorganisms in soil using denaturing gradient gel electrophoresis (DGGE)

DNA extraction

Rhizosphere soil samples (0.1–0.5 g) were subjected to DNA extraction using a Quick-DNATM Fecal/Soil Microbe Miniprep kit (Zymo Research, UK) following the manufacturer's instructions. Each sample was extracted twice using DNA extraction kit, and the resulting DNA extracts were pooled together. The genomic DNA of each sample was eluted with 60 µL TE buffer. Subsequently, genomic DNA (3 µL) was mixed with loading dye (2 µL) and loaded into the wells prepared from a 1.5% (w/v) agarose gel. Electrophoresis was performed with a 1.5% (w/v) agarose gel at 100 V for 1 h.

Polymerase chain reaction (nested PCR)

The 16 S rRNA genes were amplified using the extracted DNA as a template in PCR amplification conducted with a FlexCycler2 PCR thermal cycler (Analytix Jena, Germany). The primers utilized in this study were the

8 F primer (5'-AGA GTT TGA TCM TGG CTC AG-3') and the 1512R primer (5'-ACG GYT ACC TTG TTA CGA CTT-3')[72,73]. The PCR conditions were as follows: pre-denaturation at 95 °C for 10 min, denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1.3 min, and a final extension at 72 °C for 10 min. PCR products were visualized using gel electrophoresis. The 16 S rRNA was then subjected to the V3 region step reaction with the Forward primer 318 F (GC-clamp) (5'-ACTCCTACGGGAGGCAGCAG-3') and the Reverse primer 518R (5'-ATTACCGCGGCTGCTGG-3')[30], with PCR consisting of the following steps: pre-denaturation at 94 °C for 10 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and final extension at 72 °C for 7 min. PCR products were detected by DNA band visualization using gel electrophoresis (Amresco, USA).

DGGE

The V3 region of 16 S rRNA genes was analyzed by the DGGE technique, which involved 40–60% urea-formamide denaturing gradients. Subsequently, 20 µl of each DNA sample was loaded onto well of a 1.5% (w/v) agarose gel. The gel was run in 1X TAE buffer at 90 V and a temperature of 60 °C for 8 h. Following electrophoresis, the gel was stained with ethidium bromide (10 µg/ml) to visualize the DNA bands under UV light (QUANTUM-ST4 1100/26MX, France).

Statistical analysis

One way ANOVA analysis was performed on the data. Post-hoc analysis, such as Tukey's HSD, was conducted to identify which specific treatments differed significantly. Statistical significance was set at $p < 0.05$, and all analyses were carried out using STATISTIC 10© program (1985–2013) (Analytical Software, Tallahassee, FL, USA). This separation was performed at both the 95% and 99% confidence levels.

Data availability

All data in this study are available upon request from the corresponding author at nunrid@kku.ac.th.

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Methodology, analysis, N.R, Y.N.M and P.S; writing-original draft, N.R and Y.N.M.; funding acquisition, P.T. All author (N.R., Y.N.M., P.S., and P.T.) discussed the result.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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