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Research

Enhanced Rice (*Oryza sativa* L.) Plant Growth and Nutrient Contents During The Vegetative Stage Through Zinc Solubilizing Bacterial Bead Inoculation

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ABSTRACT

Zinc-solubilizing bacteria (ZSB) can increase zinc bioavailability in soil and transform insoluble zinc into an accessible form, which helps reduce crop zinc deficiencies, simultaneously improving soil fertility and crop nutrition. The effects of two ZSB strains, *Acinetobacter nosocomialis* (SR R-10) and *Acinetobacter seifertii* (SR-12) were evaluated in the present study on the rice plant growth and nutrient contents using the bead inoculation method. A completely randomized design (CRD) was employed and four treatments were applied: 1) non-inoculated (control), 2) SR R-10 strain, 3) SR R-12 strain, and 4) mixed inoculation of SR R-10 and SR R-12 strains. After 40 days of sowing, the growth parameters were measured. The results revealed that SR R-10 inoculant enhanced the growth by producing the tallest plant (63.47 ± 1.87 cm) and longest root (19.93 ± 0.48 cm). SR R-10-treated plants also showed the highest leaf count (32 ± 0.58 leaves) and Soil Plant Analysis Development (SPAD) value (32.67 ± 1.59). The mixed inoculant showed synergistic benefits, indicated by the higher plant height, SPAD reading, and leaf count, compared to the non-inoculated treatment. SR R-10 and mixed inoculant increased plant biomass, measuring 4.67 ± 0.30 g and 4.40 ± 0.28 g, respectively, compared to non-inoculated plants (3.19 ± 0.17 g). For nutrient content, plants with SR R-10 inoculation showed the highest concentration of nitrogen (2.24 ± 0.00%), phosphorus (0.24 ± 0.00%), potassium (2.79 ± 0.03%), and zinc (59.51 ± 2.69 mg kg^{-1}). Mixed inoculant also improved soil fertility by increasing the available Zn (6.17 mg kg^{-1}) in the soil, however, it lowered the soil pH to pH 5.8. These findings highlight the potential of ZSB, particularly *A. nosocomialis* (SR R-10), to improve rice plant's growth and nutritional quality and increase the bioavailability of zinc in the soil to promote sustainable agricultural practices.

Key words: *Acinetobacter* sp., *Oryza sativa* L., sustainable approach, zinc-solubilizing bacteria, zinc deficiency

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INTRODUCTION

Zinc is an essential micronutrient for plants, which plays a crucial role in regulating enzymatic activity as a cofactor, promoting photosynthesis by forming the chlorophyll and synthesis of plant hormones such as auxin and gibberellin. These hormones are important in plant vital processes such as fruit and flowering development as well as root and stem elongations. Zinc is also required for various essential metabolic activities in plants, including protein synthesis, glucose metabolism, and nucleic acid synthesis (Fageria & Santos, 2014; Hafeez *et al*., 2015; Suganya *et al*., 2020).

Zinc in soils exists in five distinct pools: solution or water-soluble, adsorbed or exchangeable, organic matter-associated, oxides and carbonates-associated, and zinc in weathered primary minerals (Alloway, 2008; Banasode

& Channakeshava, 2022). Zinc deficiency has become the most widespread micronutrient issue in agriculture worldwide (Gandhi & Muralidharan, 2016; Kandil *et al*., 2022). Low-zinc soil is attributed to the low total zinc concentrations in the soil, or it may have high concentrations but be less available to the plants due to the insoluble forms of zinc (Rengel, 2015). For better plant growth and yield, zinc fertilizer needs to be applied to the soil with low plant-available zinc (Hafeez *et al*., 2013; Liu *et al*., 2020).

Kedah and Kelantan are Malaysia's main areas for rice cultivation (Department of Agriculture Malaysia, 2018). However, it has been reported that the soils in these places have low plant-available zinc, resulting in low zinc levels in plants. Zinc levels decrease with soil depth. Thus, it is recommended that both organic and inorganic fertilizers be applied (Hafeez *et al*., 2015). Zinc sulfate and zinc oxide are the rice producer's most common inorganic zinc fertilizers. However, zinc oxide is less soluble than zinc sulfate (Gangloff *et al*., 2006). After 15 days of application, zinc sulfate, which is soluble in water, was also shown to be unavailable and this might be because the soil equilibrium caused the released zinc to transform into insoluble forms (Sunitha *et al*., 2014; Rengel, 2015; Rudani *et al*., 2018). A buildup of insoluble zinc salts in soils may destroy beneficial soil microbes, contaminate underground water, and further reduce soil fertility, which can alter plant growth and development (Rutkowska *et al*., 2015; Shakeel *et al*., 2015; Shukla *et al*., 2016; Kaur & Garg, 2021).

Applying zinc-solubilizing bacteria is one viable and promising approach to overcoming this issue (Sindhu *et al*., 2019). Several zinc-solubilizing bacteria, such as *Bacillus altitudinis (Kushwaha et al., 2021), Bacillus megaterium* (Bhatt & Maheshwari, 2020) and *Pseudomonas protegens* RY2 (Yasmin *et al*., 2021), have been identified as plant growth-promoting bacteria due to their abilities to make zinc accessible to plants. These bacteria can solubilize the unavailable zinc form by secreting organic acids, producing chelating ligands and phytohormones. Using a microbial inoculant is the best alternative to chemical fertilizer and helps to maintain soil fertility (Sunitha *et al*., 2014). Besides, alginate beads can be used as carriers, which provide a slow and constant release of bacteria (Rocha *et al*., 2019).

Two strains of zinc-solubilizing bacteria, *Acinetobacter nosocomialis* (SR R-10) and *Acinetobacter seifertii* (SR-12) were used in this study. To our knowledge, no previous studies have reported the development of microbeads carrying *Acinetobacter* strains and their impact on rice plant growth. In our previous study, we screened several potential isolates, and the results showed that these strains, *A. nosocomialis* (SR R-10) and *A. seifertii* (SR-12), can effectively solubilize zinc in various insoluble forms, including zinc oxide (ZnO), zinc carbonate ($ZnCO_3$), and zinc phosphate ($Zn_3(PO_4)_2$) (Othman *et al*., 2022; Irsyad *et al*., 2023). These results demonstrate that these strains are excellent candidates for further assessment in our present study.

MATERIALS AND METHODS

Preparation of potting soil and rice seed

The plastic pots used in this study were round (15 cm in diameter) × 14 cm in height). The soil used in the experiment was collected from a depth of 20 cm in a rice field located in Bukit Rambai, Malacca, Malaysia (coordinate: 2°15'45"N, 102°9'9"E). About 10 g of air-dried soil samples were weighed and ground to pass through a 2-mm sieve. The initial physicochemical properties of the soil were determined as shown in Table 1. For the seed, MR 219 Malaysian rice variety (*Oryza sativa* L., accession number: MRGB11633) was obtained from the Malaysian Agricultural Research and Development Institute (MARDI), Seberang Prai, Pulau Pinang. The seeds were sterilized by treating them with sodium hypochlorite solution for about 10 min and washed with sterile distilled water several times. Then, the seeds were soaked in sterile distilled water for 24 hr. Sprouted rice seeds were then ready to be planted in the prepared pots where each pot consisted of three seeds (Syaziana *et al*., 2024).

Table 1. Physicochemical properties of the soil used in the study

Properties	Values
Type	Alluvium
pH	6.42
Nitrogen	0.19%
Phosphorus	5.04 mg kg^{-1}
Potassium	76.70 mg kg^{-1}
Zinc	4.82 mg kg^{-1}
Calcium	3.98%

Source of zinc-solubilizing bacteria (ZSB)

Two zinc-solubilizing bacteria (ZSB) strains, *Acinetobacter nosocomialis* (SR R-10) and *Acinetobacter seifertii* (SR R-12) were used as inoculums. These strains were obtained from the Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Melaka, Malacca, Malaysia. These isolates were previously isolated, identified, and characterized in our preliminary study from the rice rhizosphere soil (Irsyad *et al*., 2023). The descriptions of the strains used are shown in Table 2.

Table 2. The descriptions of the ZSB strains (SR R-10 & SR R-12) used in the study

Description	SR R-10	SR R-12
Scientific name	*Acinetobacter nosocomialis*	*Acinetobacter seifertii*
Source	Rice rhizoplane	Rice rhizoplane
NCBI accession number	ON834324	ON954519
Public deposit (UPM UNiCC)	UPMC 1417	UPMC 1418
Characteristics	Zinc solubilizer, nitrogen fixer, phosphate solubilizer, potassium solubilizer, siderophores and indole compounds producer	Zinc solubilizer, nitrogen fixer, phosphate solubilizer, potassium solubilizer, siderophores and indole compounds producer

Encapsulation of zinc-solubilizing bacteria (ZSB)

For encapsulation, the bacterial cultures of both ZSB strains (10^8 to 10^9 CFU) were first mixed with 2% sodium alginate under aseptic conditions (Syaziana *et al*., 2024). The mixtures were then gently titrated in 0.1 M of calcium chloride using a sterile syringe. The beads were formed and sank immediately to the bottom of the solution and maintained at room temperature for 2 hr. The well-formed beads were washed a few times with sterile distilled water before being kept in a sterile glass bottle and chilled.

Experimental design

This experiment used a completely randomized design (CRD) (Syaziana *et al*., 2024). Four treatments were applied to the rice plants: 1) non-inoculated (control), 2) single strain of SR R-10, 3) single strain of SR R-12 and 4) mixed inoculation of both bacterial strains (SR R-10 & SR R-12). The control group consisted of rice seedlings grown without the microbeads. All treatments were assigned four replicates with a total of 16 pots with three rice seedlings per pot. Zinc oxide (Merck, United States) was applied to each pot at a fixed rate of 100 mg kg^{-1} of soil to produce high Zn content in the soil but low plant-available fraction conditions (Gandhi & Muralidharan, 2016). Both bacterial strains used as the inoculum were first encapsulated in the form of microbeads. About 6.60 g of ZSB beads (2.20 g of beads per plant) were applied evenly on the soil surface within the topsoil (about 0-15 cm of depth) for each treatment pot with inoculation (SR R-10, SR R-12 & mixed). Microbeads were applied at a 1:1 ratio for treatment with mixed inoculation. The treatment was applied as a single application one week post-sowing, with no subsequent treatments conducted throughout the rice growth cycle.

Measurement of plant growth parameters

Plant height, root length, root weight, biomass, leaf greenness, and number of leaves were recorded as rice growth parameters (Syaziana *et al*., 2024). Plant height was measured from the base of the plants, excluding the roots, to the tallest shoots (Zakaria *et al*., 2017). The root length was taken after the root part had been washed, followed by recording the fresh weight of the roots. The roots were then dried at 60°C for a few days and recorded for their dry weight. Leaf greenness was assessed using a Soil Plant Analysis Development (SPAD) meter (Konica Minolta, Japan) by taking average readings from the fully expanded topmost leaf of each rice plant, with three leaves sampled per plant. A high reading on the SPAD meter means the leaves have a high chlorophyll content since they need more light to get through them. The number of leaves was recorded by directly counting the leaves per pot. All the growth parameters were taken 40 days after sowing (DAS) because it represents the rice vegetative growth period.

Analysis of nutrient contents

For the nutritional analysis, a whole rice plant was used (Izilan *et al*., 2022). The total nitrogen content was determined by using the combustion method. The plant tissue samples (10 mg) were weighed in tin containers and loaded into the automatic sampler. Then, total nitrogen content was measured by gas chromatography using a CNS analyzer (LECO 928 Series, Germany). While for other parameters such as phosphorus (P), potassium (K), zinc (Zn), and iron (Fe), the samples were first oven-dried at 60°C overnight. Then, the samples were digested by using the dry ashing method, and nutrient concentrations were measured by using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) Optima 7300DV (PelkinElmer, Inc, Waltham, MA, USA).

Analysis of soil properties

Soil pH was determined using the soil-water ratio method (1:2.5) (Al-Busaidi *et al*., 2005). Soil samples were taken from all 16 plots which represent four replicates for four treatments in this study. The soil samples were sampled from top soil which was 0-15 cm. Ten g of soil samples were collected and added to 25 mL of distilled water. The samples were then shaken for five min at 180 rpm by using a reciprocating shaker. The samples were allowed to settle for a few min before being measured for acidity using a pH meter (Mettler Toledo, United States). 25 mL of Mehlich No. 1 extracting reagent (0.05 M HCl in 0.025 N H_2SO_4) was added to 5 g of soil sample in the plastic vial to determine zinc concentration. The sample was shaken for about 5 min on a reciprocating shaker at 180 rpm before being filtered using Whatman No. 2. The extract was analyzed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) Optima 7300DV (PelkinElmer, Inc, Waltham, MA, United States) (Izilan *et al*., 2022).

Statistical analysis

The data were tested for normality and homogeneity of variance before being analyzed using one-way ANOVA, and significance differences between means were compared using post hoc Tukey's HSD at 5% probability using SPSS software (version 28.0.0.0).

RESULTS

Rice plant growth

The rice plant growth parameters were recorded in Table 3. Although no significant differences were observed, the results indicated improvements in plant height, root length, SPAD readings, and the number of leaves following the inoculation of ZSB. Treatments with ZSB inoculation showed higher plant height (63.47 ± 1.87 cm) by *A. nosocomialis* (SR R-10) followed by *A. seifertii* (SR R-12) and mixed inoculation (SR R-10 + SR R-12), with both recorded heights at 61.57 ± 1.29 and 59.63 ± 1.19 cm, respectively, at $p<0.05$ (Figure 1). Meanwhile, the root of the inoculated treatments was also longer as compared to the non-inoculated, as shown in Figure 2. The inoculation of *A. nosocomialis* (SR R-10) showed the longest root length at 19.93 ± 0.48 cm. There was no significant difference recorded for root dry weight.

Table 3. Effects of ZSB inoculation on the growth of rice plant

Treatment	Height (cm)	Root length (cm)	Root dry weight (g)	Biomass (g)	SPAD reading	Number of leaves
Non-inoculated	53.80 ± 0.27^b	16.17 ± 0.20^c	0.75 ± 0.05^a	3.19 ± 0.17^b	23.57 ± 0.27^c	26.67 ± 0.33^c
A. nosocomialis (SR R-10)	63.47 ± 1.87^a	19.93 ± 0.48^a	0.88 ± 0.02^a	4.67 ± 0.30^a	32.67 ± 1.59^a	32.00 ± 0.58^a
A. seifertii (SR R-12)	61.57 ± 1.29^a	18.30 ± 0.40^b	0.85 ± 0.04^a	4.01 ± 0.07^{ab}	30.20 ± 0.59^b	29.33 ± 0.88^b
Mixed (SR R-10 + SR R-12)	59.63 ± 1.19^a	18.80 ± 0.29^b	0.87 ± 0.02^a	4.40 ± 0.28^a	27.47 ± 0.12^b	30.67 ± 0.33^{ab}

Values are means ± standard error. Values with the same letters in each parameter column among the treatments are not significantly different according to Tukey's HSD at $p=0.05$.

For plant biomass, a higher biomass was only observed for the inoculation with *A. nosocomialis* (SR R-10) and mixed inoculation (SR R-10 + SR R-12) at $p<0.05$. Inoculation of *A. nosocomialis* (SR R-10) and mixed inoculation (SR R-10 + SR R-12) showed higher plant biomass compared to the other treatments at $p<0.05$, with both recording 4.67 ± 0.30 and 4.40 ± 0.28 g, respectively. However, plant biomass obtained through the inoculation of *A. seifertii* (SR R-12) at 4.01 ± 0.01 g was not higher than the non-inoculated treatment, which only produced 3.19 ± 0.17 g of biomass.

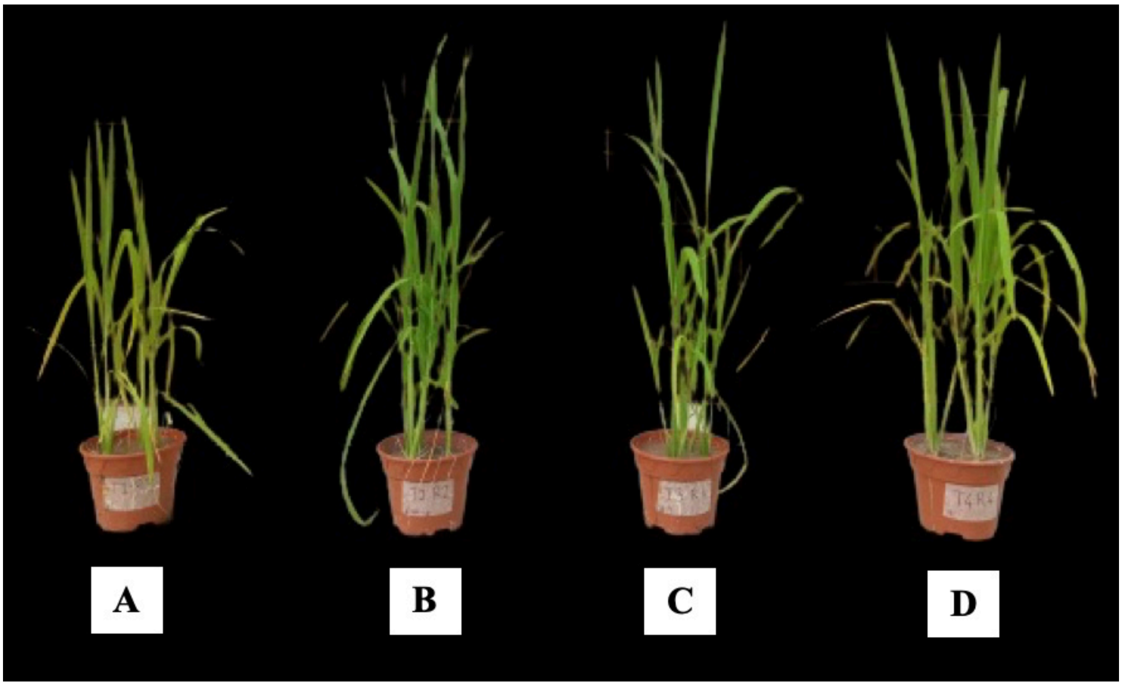

Fig. 1. Height of rice plants at 40 days after sowing. From left to right: (A) non-inoculated; (B) *A. nosocomialis* (SR R-10); (C) *A. seifertii* (SR R-12); (D) mixed inoculation (SR R-10 + SR R-12).

The highest reading for chlorophyll SPAD meter was obtained by the treatment with inoculation of *A. nosocomialis* (SR R-10) at 32.67 ± 1.59, followed by *A. seifertii* (SR R-12) at 30.20 ± 0.59, and mixed inoculation (SR R-10 + SR R-12) at 27.47 ± 01.2 at $p<0.05$. As for the number of leaves, all treatments with inoculation were higher than non-inoculated treatments. Treatment with *A. nosocomialis* (SR R-10) recorded the highest number, with 32 leaves produced compared to other treatments.

Fig. 2. Root length of rice plants at 40 days after sowing. From left to right: (A) non-inoculated; (B) mixed inoculation (SR R-10 + SR R-12); (C) *A. seifertii* (SR R-12); (D) *A. nosocomialis* (SR R-10).

Nutritional analysis of rice plant

Table 4 indicates that the analysis of nutrient contents did not show statistically significant differences between the treatment groups. However, the treatments involving ZSB inoculation exhibited higher concentrations of nitrogen (N), phosphorus (P), potassium (K), and zinc (Zn) in the rice leaves than in the non-inoculated treatments. Meanwhile, for Fe concentrations, all inoculated treatments have lower concentrations than non-inoculated ones. The highest nitrogen concentrations were obtained through the inoculation of single strain *A. nosocomialis* (SR R-10) at 2.24 ± 0.00%, followed by single strain *A. seifertii* (SR R-12) at 1.94 ± 0.00%, and mixed inoculation (SR R-10 + SR R-12) at 1.76 ± 0.00% of N.

For phosphorus and potassium, treatment with inoculation of *A. nosocomialis* (SR R-10) recorded 0.24 ± 0.00% for P and 2.79 ± 0.03% for K as compared to the non-inoculated treatment, which recorded 0.21 ± 0.00% for P and 2.55 ± 0.01% for K. Inoculation of *A. nosocomialis* (SR R-10) obtained the highest Zn content (59.51 ± 2.69 mg kg^{-1}) followed by mixed inoculation (SR R-10 + SR R-12) and *A. seifertii* (SR R-12) as both recorded 51.27 ± 0.49 and 48.96 ± 0.50 mg kg^{-1} of Zn, respectively. The rice plant without ZSB inoculation had the highest iron concentration in tissue, as it recorded 191.530 ± 1.48 mg kg^{-1} compared to the lowest 146.937 ± 1.37 mg kg^{-1} obtained by the treatment with inoculation of *A. nosocomialis* (SR R-10).

Table 4. Effects of ZSB inoculation on the nutrient contents in the rice plant tissue

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Zinc (mg kg^{-1})	Iron (mg kg^{-1})
Non-inoculated	1.67 ± 0.00^{d}	0.21 ± 0.00^{c}	2.55 ± 0.01^{c}	42.58 ± 0.33^{c}	191.53 ± 1.48^{a}
A. nosocomialis (SR R-10)	2.24 ± 0.00^{a}	0.24 ± 0.00^{a}	2.79 ± 0.03^{a}	59.51 ± 2.69^{a}	146.94 ± 1.37^{c}
A. seifertii (SR R-12)	1.94 ± 0.00^{b}	0.22 ± 0.00^{b}	2.63 ± 0.01^{b}	48.96 ± 0.50^{b}	169.53 ±2.62^{b}
Mixed (SR R-10 + SR R-12)	1.76 ± 0.00^{c}	0.22 ± 0.00^{b}	2.76 ± 0.01^{a}	51.27 ± 0.49^{b}	153.24 ± 4.20^{c}

Values are means ± standard error. Values with the same letters in each parameter's column

Analysis of soil nutrient

The statistical analysis showed no significant differences between treatments. However, all inoculated treatments exhibited lower pH levels compared to the non-inoculated treatment. Treatment with inoculation of *A. nosocomialis* (SR R-10) showed the lowest soil pH at 5.79, followed by *A. seifertii* (SR R-12) and mixed inoculation (SR R-10 + SR R-12) as both recorded soil pH at 5.80 (Figure 3). While the non-inoculated treatment showed the highest pH value (5.93) at p<0.05. Soil available Zn concentration was affected by treatments with mixed inoculation (SR R-10 + SR R-12) and *A. nosocomialis* (SR R-10), as shown in Figure 4. Treatment with mixed inoculation (SR R-10 + SR R-12) recorded 6.17 mg kg^{-1} Zn, while inoculation with *A. nosocomialis* (SR R-10) recorded 5.37 mg kg^{-1} Zn. The non-inoculated rice plant obtained the lowest soil available Zn concentration (4.74 mg kg^{-1}) at p<0.05.

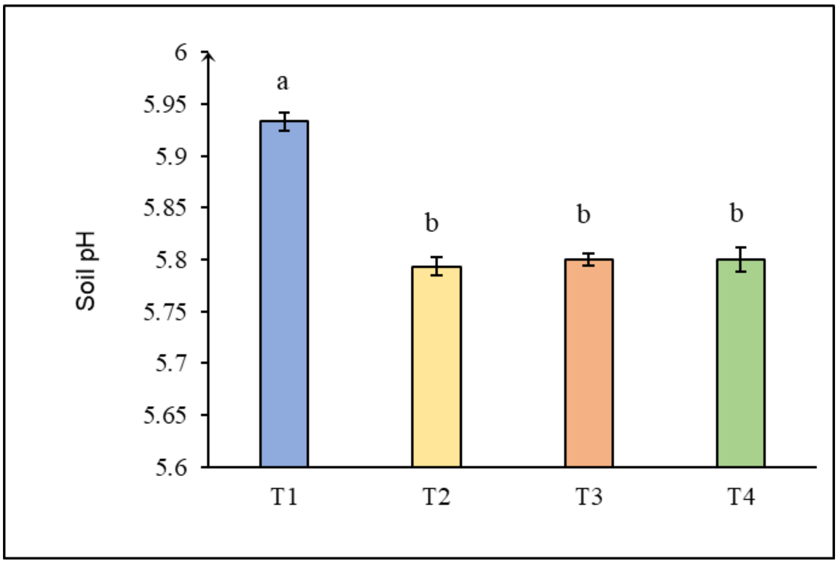

Fig. 3. Soil pH at 40 days after planting with rice. T1 = non-inoculated (control), T2 = *A. nosocomialis* (SR R-10), T3 = *A. seifertii* (SR R-12), T4 = mixed inoculation (SR R-10 + SR R-12). Values are means ± standard error. Error bars refer to the standard error. Values with the same letters are not significantly different according to Tukey's HSD at p=0.05.

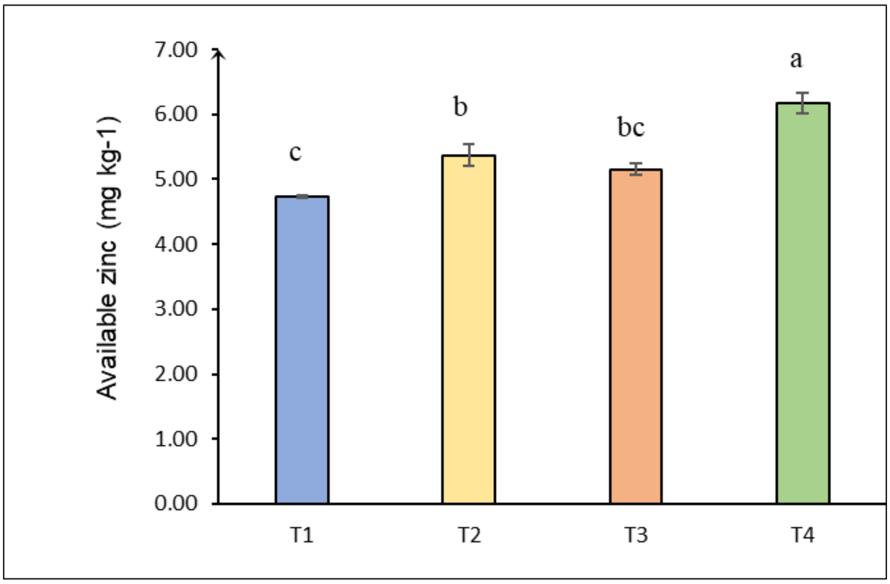

Fig. 4. Soil available zinc 40 days after planted with rice. T1 = non-inoculated (control), T2 = *A. nosocomialis* (SR R-10), T3 = *A. seifertii* (SR R-12), T4 = mixed inoculation (SR R-10 + SR R-12). Values are means ± standard error. Error bars refer to the standard error. Values with the same letters are not significantly different according to Tukey's HSD at p=0.05.

DISCUSSION

The cultivation of the rice plants was carried out up to 40 DAS, focusing on the vegetative phase of the rice growth cycle. This limitation was imposed by the plant's specific need for zinc nutrients during this critical phase. Other than that, during the vegetative phase of rice growth, the bacteria exhibited heightened activity, particularly under submerged conditions. This phase is crucial as it corresponds to the early stages of rice development, where the availability of both macro- and micronutrients plays a vital role in supporting healthy plant growth (Pittol *et al*., 2018).

Numerous bacteria species from the genera *Acinetobacter, Enterobacter, Bacillus, Klebsiella, Ralstonia, Serratia, Gluconacetobacter, Burkholderia,* and *Pseudomonas* were beneficial in nutrient solubilization and were able to revert the insoluble fraction of nutrient into soluble form (Gandhi *et al*., 2014; Dinesh *et al*., 2018; Jiang *et al*., 2020; Haroon *et al*., 2022). However, in vitro studies are important to test their effectiveness, as many bacterial isolates presenting superior plant growth-promoting characteristics under in vitro conditions often fail to maintain the same level of efficacy under realistic conditions when challenged by native microorganisms (Kamran *et al*., 2017).

From our findings, the growth of rice plants was significantly enhanced by the inoculation of ZSB as the growth parameters observed in the inoculated treatment showed improvements compared to the non-inoculated treatment. The significant increase in rice height across all inoculation treatments further demonstrates the positive influence of ZSB on rice plant growth. Height is often used to indicate plant vigor and overall health, suggesting that the presence of ZSB has facilitated improved physiological activities within the rice plants. Similar findings were also reported in the previous studies using different species of bacteria strains. For example, rice treated with isolate OS07, a phosphate solubilizing bacteria, and supplemented with triple super phosphate was reported to increase the plant height compared to rice without bacterial inoculation (Sarkar *et al*., 2012).

In another study, the combination of two zinc fertilizers (zinc oxide & zinc carbonate) with two bacterial isolates (AGM3 and AGM9) recorded the highest rice height (75.08 cm) after 150 DAS (Gandhi & Muralidharan, 2016). A significant impact of bacteria inoculation was also found on *Capsicum annuum* L., as reported by Bhatt and Maheswari (2020), where treatment with *B. megaterium* (CDK25) recorded the highest plant height (27.84 cm), stem girth (15.32 mm), and vigor index (1855.81%) at 60 DAS. These showed that the effectiveness of bacteria inoculation on increasing rice height during the vegetative phase could have further benefits until the ripening phase.

Other parameters such as root length, biomass, leaf greenness, and number of leaves of inoculated rice plants were also significantly higher than those of non-inoculated plants. Previous studies found that inoculating wheat with *Enterobacter cloacae* (PBS 2) increased root length, and similar results were seen with ZSB strains like *Bacillus* sp. (ZM20), *Bacillus aryabhattai* (ZM31 & S10), and *Bacillus subtilis* (ZM63) (Kamran *et al*., 2017; Mumtaz *et al*., 2017). Shakeel *et al*. (2015) found that when rice varieties basmati-385 and super basmati were treated with *Bacillus* zinc solubilizers, their leaves were greener than those without the bacteria or with only zinc treatment. Similarly, Jha (2019) observed that the chlorophyll content in rice variety GJ17 increased significantly when treated with *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus*, especially under salt stress, compared to the untreated plants.

Vaid *et al*. (2014) found that inoculation of ZSB (genus *Burkholderia* and *Acinetobacter*) individually or in combination was able to increase the dry matter of rice by 12.9% and the number of tillers by 15.1% over the control and zinc fertilizer treatments. However, their findings also showed that not all combinations of ZSB may produce positive effects, as some of the bacterial isolate combinations such as AX+AB isolates, were significantly lower in the mean dry matter yield compared to the individual treatment of AX isolate. Bhatt and Maheswari (2020) also found that treatment with a combination of CDK15 and CDK25 isolates on *Capsicum annuum* L. was less effective than a single treatment with CDK25 in enhancing plant growth and this was in line with our findings, as we found treatment with SR R-10 produced better rice growth than the combination of SR R-10 with SR R-12. It can be assumed that under certain conditions, a single inoculation of bacteria is more effective than a combination of bacteria. Factors such as competition for resources, compatibility of metabolic pathways, and microbial community dynamics can influence the overall effectiveness of consortium treatments.

Previous studies also reported the positive effects of ZSB on increasing the nutrient content in plant tissue. In the study by Kamran *et al*. (2017), zinc content in wheat shoots and roots increased for inoculated plants after four weeks, suggesting that ZSB enhanced the bioavailability of zinc and mobilized it toward wheat grains. ZSB not only helps in zinc deficiency, but can also facilitate the absorption of other nutrients into plant tissues, including nitrogen, potassium, and phosphorus (Singh *et al*., 2024). The current investigation shows that the levels of these nutrients are elevated in the ZSB-inoculated treatments. However, the iron levels in rice leaves were observed to be reduced through ZSB inoculation. This can be attributed to the iron-zinc interaction as a negative correlation (Hafeez *et al*., 2013).

Previous studies have reported positive correlations between SPAD readings, chlorophyll, and nitrogen concentrations, specifically in rice (Singh *et al*., 2002; Hou *et al*., 2020). The nitrogen content in the current study is consistent with the SPAD readings. This connection indicates a relationship between ZSB inoculation, nitrogen availability, and plant health. ZSB can facilitate nitrogen fixation, making it more available to plants. Consequently, plants may absorb more nitrogen, resulting in higher nitrogen levels in their tissues. Because nitrogen is an important component of chlorophyll, increasing nitrogen levels can lead to improved leaf greenness, as seen by SPAD values.

In this study, only pH and zinc were analyzed for soil because the study focused on the role of zinc-solubilizing bacteria in enhancing zinc availability in the soil, which explains the only soil nutrient analysis focused on zinc. However, for plant growth parameters, both micro and macronutrients were measured because zinc uptake might influence the overall nutrient balance and concentration in plants. According to Boguta and Sokolowska (2020), Zn activity in the soil correlates directly with rising proton activity; hence, Zn solubility is always inversely proportional to soil pH and therefore the solubility and mobility of Zn was higher in acidic soil than in alkaline, indicating that the pH of the soil is an important factor for Zn availability. In our study, we found that the rice plant treated with SR R-10 and mixed inoculant (SR R-10 + SR R-12) lowered the soil pH compared to those without inoculation, while also increasing the zinc concentration in the soil. This finding is supported by Dinesh *et al*. (2018) who found that the inoculation of *B. megaterium* (ZnSB2) decreased the soil pH, which might be attributed to the bacteria's enhanced production of gluconic acid. Bacterial synthesis of organic acids reduces soil pH, hence increasing nutrient solubility in the soil (Rutkowska *et al*., 2015; Vidyashree *et al*., 2018).

CONCLUSION

This study concluded that the inoculation of ZSB strains, *A. nosocomialis* (SR R-10) and *A. seifertii* (SR R-12), show potential as bio-inoculants. Inoculating these strains individually resulted in better plant growth and nutrient content compared to mixed inoculation (SR R-10 + SR R-12), although no significant differences were found between the treatments. However, the mixed inoculation treatment performed better than the others in terms of zinc availability in the soil. ZSB addressed Zn deficiencies and improved the uptake of other essential nutrients like phosphorus, nitrogen, and potassium. Concisely, the inoculation of ZSB in slow-released microbeads can be used as an environment-friendly approach for improving plant development and soil health in sustainable agriculture.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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