



RESEARCH ARTICLE

# Synergistic effects of rhizospheric and nodule associated zinc solubilising consortia to enhance productivity and biofortification in lentil (*Lens culinaris* L.)

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## Abstract

The present study was undertaken to isolate, screen and evaluate zinc-solubilising bacteria (ZnSB) from lentil rhizosphere and root nodules and to assess their individual and consortium effects on plant growth, yield, micronutrient biofortification and soil nutrient status. A total of 120 rhizospheric soil and 62 root samples were collected from RPCAU research farms at Pusa and Dholi, yielding 157 bacterial isolates. Among these, 127 rhizospheric and 30 nodule isolates were screened for zinc solubilisation using zinc oxide (ZnO) and zinc carbonate (ZnCO<sub>3</sub>) as zinc sources. The ZnO was the most preferred substrate, with Zn solubilisation efficiency (Zn-SE) ranging from 25.00 to 416.70 in rhizospheric (Rh) isolates and 57.10 to 357.10 in nodule (Nd) isolates. The most efficient isolates (Rh-ZnSB 38, Rh-ZnSB 60, Rh-ZnSB 90 and Nd-ZnSB 1) also exhibited multiple plant growth-promoting traits, including phosphate and potassium solubilisation, siderophore production, indole-3-acetic acid (IAA) and gibberellic acid (GA) synthesis and ammonia production and were found to be highly compatible with each other. Based on these traits, a microbial consortium was developed. Seed germination studies revealed that combined inoculation of Nd-ZnSB 1 with the Rh-ZnSB consortium resulted in 100 % germination and the highest seed vigour index, indicating strong synergistic effects. Pot experiments further demonstrated that ZnSB consortia significantly enhanced plant growth and nodulation. Plant height ranged from 30.60–38.80 cm at 45 days after sowing (DAS) and 42.10–57.90 cm at 90 DAS, while nodules varied from 18.50–38.90 plant<sup>-1</sup> at 45 DAS and 24.30–45.60 plant<sup>-1</sup> at 90 DAS, with consortium treatment T<sub>7</sub> consistently outperforming other treatments. Yield attributes were also markedly improved by ZnSB consortia. The number of pods per plant ranged from 50.00 to 75.00, seeds per plant from 69.00 to 130.00, 100-seed weight from 2.40 to 5.30 g and grain yield from 15.00 to 30.00 g pot<sup>-1</sup>, with maximum values recorded under consortium treatments. Grain biofortification was significantly enhanced, with Zn content ranging from 21.40 to 46.80 mg kg<sup>-1</sup> and iron from 62.50 to 102.30 mg kg<sup>-1</sup>. In addition, ZnSB consortia improved soil fertility by increasing available nitrogen (0.78–1.18 g kg<sup>-1</sup>), phosphorus (0.32–0.64 g kg<sup>-1</sup>), potassium (1.15–1.56 g kg<sup>-1</sup>), organic carbon (0.42–0.66 %) and DTPA-extractable Zn and Fe.

**Keywords:** compatibility, microbial consortia, plant growth-promoting rhizobacteria, *Rhizobium*, zinc-solubilising bacteria

## Introduction

Lentil (*Lens culinaris* L.) is one of the most important cool-season grain legumes cultivated worldwide and holds a prominent place in Indian agriculture. Globally, lentil is the fifth most widely cultivated legume, grown in more than 50 countries over an area of approximately 4.8 million hectares, with an annual production of about 5.73 million tonnes (1). India ranks second in global lentil production, with extensive cultivation in Uttar Pradesh, Madhya Pradesh, Jharkhand, Bihar and West Bengal. Often referred to as the “poor man’s meat,” lentil is highly valued for its rich nutritional profile, containing 20–26 % protein, balanced carbohydrates and lipids and essential micronutrients such as zinc (Zn) and iron (Fe) (2). Originating from the Fertile Crescent, lentil exhibit high digestibility, short cooking time and remarkable adaptability to diverse agro-

climatic conditions, including tolerance to frost and heat stress (3). Moreover, lentil thrives in low-fertility soils and contributes to sustainable farming systems through symbiotic nitrogen fixation with *Rhizobium* spp., thereby reducing dependence on synthetic nitrogen fertilisers and improving soil health (4).

Despite its agronomic and nutritional importance, lentil productivity and nutritional quality are often constrained by micronutrient deficiencies, particularly Zn and Fe. Zinc deficiency is a widespread problem in Indian soils, especially in Vertisols and Alfisols of Karnataka, Madhya Pradesh, Andhra Pradesh and Tamil Nadu (51–78 %), the Indo-Gangetic plains of Uttar Pradesh and Bihar (45–54 %) and leached Ultisols of Meghalaya, West Bengal and Odisha (36–57 %) (5, 6). In lentil, Zn deficiency symptoms typically appear 5–6 weeks after sowing and are characterised by chlorosis of

young leaves, followed by browning, leaf abscission, stunted growth and poor pod formation (7–9). These deficiencies are further aggravated under alkaline soil conditions with high calcium carbonate content, whereas increasing soil organic matter improves Zn availability.

To overcome nutrient constraints, farmers frequently rely on chemical fertilisers for rapid yield enhancement. However, prolonged and imbalanced fertilisers use has resulted in soil degradation, nutrient mining, disruption of beneficial soil microbial communities and declining nutrient-use efficiency (10). Furthermore, nutrient losses through runoff and leaching contribute to eutrophication of water bodies, algal blooms, oxygen depletion and loss of aquatic biodiversity (11, 12). These challenges underscore the urgent need for sustainable, eco-friendly nutrient management strategies that can enhance crop productivity while maintaining soil and environmental health.

In this context, plant growth-promoting rhizobacteria (PGPR), particularly ZnSB, have emerged as promising biological tools for sustainable agriculture. The ZnSB enhance Zn availability in soil through mechanisms such as organic acid production, chelation, proton extrusion and siderophore secretion (13, 14). When co-inoculated with *Rhizobium*, ZnSB exhibit synergistic interactions that improve Zn uptake, nodulation efficiency, biological nitrogen fixation and overall plant growth in legumes. Importantly, microbial consortia comprising two or more compatible ZNSB strains often perform better than single-strain inoculants by ensuring functional redundancy, ecological stability and sustained nutrient mobilisation (15, 16, 14). Nevertheless, despite their considerable potential, systematic studies focusing on ZNSB consortia for Zn biofortification of edible plant parts remain limited (17–19).

Beyond Zn solubilisation, ZnSB commonly possesses multiple plant growth-promoting attributes, including siderophore production (20), ammonia and hydrogen cyanide synthesis (21), exopolysaccharide production (22), phytohormone secretion such as indole-3-acetic acid (IAA) and phytase activity (23) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (24). Many ZnSB strains also solubilise phosphorus and potassium, thereby improving overall nutrient availability and enhancing crop growth and yield (25, 26).

Accumulating evidence highlights the synergistic role of *Rhizobium* and PGPR consortia in improving legume productivity, nutrient uptake and grain biofortification. Previous researchers demonstrated that co-inoculation of selected *Rhizobium* strains with PGPR significantly enhanced indole-3-acetic acid (IAA), siderophore and hydrogen cyanide (HCN) production along with Zn and P solubilisation, resulting in improved lentil growth and yield (27). Similarly, another researchers reported that a multi-strain microbial consortium markedly improved germination, nodulation, biomass accumulation, yield and N, P, K, Fe and Zn enrichment across diverse soil systems, with the most pronounced effects observed in alluvial soils (28). Comparable benefits of microbial consortia have also been reported in soybean, chickpea, wheat and sugarcane systems, where enhanced Zn solubilisation, phytohormone production, nodulation and grain biofortification were consistently observed (29–33).

Based on these findings, we hypothesize that a functionally compatible consortium of ZnSB and *Rhizobium* strains isolated from leguminous crop rhizospheres will outperform individual inoculants

and conventional fertiliser practices in enhancing lentil growth, yield and grain Zn biofortification. Specifically, such a consortium is expected to accelerate seed germination, improve nodulation and biomass accumulation, enhance nutrient uptake and significantly increase Zn concentration in lentil grains, thereby contributing to sustainable lentil production and nutritional security.

## Materials and Methods

### Sampling, enrichment and isolation from rhizosphere and root nodule

Soil samples and root nodules from the rhizosphere of pulse crops, particularly lentil (*L. culinaris*), were collected from various locations across the RPCAU University farm, Pusa, Bihar ( $\approx 25.980^\circ \text{N}$ ,  $85.670^\circ \text{E}$ ) (Supplementary Table 1; Supplementary Table 2). As Bihar is a major lentil-producing region of India, the collected samples were considered representative of the local agro-ecosystems. Rhizospheric soil samples and surface-sterilised root nodules were enriched separately in King's B broth and Yeast Extract Mannitol Broth (YEMB), respectively (34). Following incubation for 48 h at  $28 \pm 2^\circ \text{C}$  under shaking conditions (120 rpm), aliquots from the enriched cultures were serially diluted and spread-plated onto King's B agar and Yeast Extract Mannitol Agar (YEMA) for bacterial isolation. Individual bacterial colonies were selected based on distinct morphological characteristics, including colony shape, size, color, margin, elevation, exopolysaccharide (EPS) production and Gram reaction (Supplementary Table 3). Selected colonies were repeatedly re-streaked on their respective media until morphologically uniform and pure cultures were obtained.

### Screening for zinc solubilisation

All rhizobacterial and nodule isolates were evaluated for their Zn-solubilising ability using Tris minimal agar medium amended with insoluble Zn sources, namely zinc oxide (ZnO) and zinc carbonate ( $\text{ZnCO}_3$ ), following the method described by previous researchers (35). Pure cultures of each isolate were spot-inoculated onto Tris minimal agar plates and incubated at  $30 \pm 2^\circ \text{C}$  for 7–10 days. The formation of clear halo zones around bacterial colonies indicated Zn solubilisation. The zinc solubilisation index (Zn-SI) and zinc solubilisation efficiency (Zn-SE) were calculated using standard formulae. The results are presented in Supplementary Tables 4 and 5 and illustrated (Fig. 1a).

Zn-SI =

$$\frac{\text{Colonydiameter (mm)} + \text{Halozone diameter (mm)}}{\text{Colonydiameter (mm)}}$$

$$\text{Zn-SE \%} = \frac{\text{Halozone diameter}}{\text{Colonydiameter}} \times 100$$

Quantitative estimation of Zn solubilisation was performed only for bacterial isolates that exhibited superior Zn solubilisation under qualitative screening. For quantitative analysis, selected isolates were inoculated into 150 mL conical flasks containing 50 mL of Tris minimal broth supplemented with 0.1 % (w/v) ZnO or  $\text{ZnCO}_3$  as insoluble Zn sources and incubated at  $30 \pm 2^\circ \text{C}$  under shaking conditions (150 rpm) for 3, 5, 7 and 11 days. Uninoculated Tris minimal medium served as the control. At each incubation interval,

5 mL of culture was aseptically withdrawn, filtered through whatman No. 1 filter paper to remove undissolved Zn particles and centrifuged at 10000 rpm for 20 min. The resulting supernatant was collected and digested using a di-acid mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$  (1 : 4, v/v). The concentration of soluble Zn in the digested samples was determined using an atomic absorption spectrophotometer (AAS). Zinc concentration was quantified against standard calibration curves prepared using ZnO and  $\text{ZnCO}_3$  and expressed as  $\mu\text{g Zn mL}^{-1}$  (Table 1), following the method described by earlier researchers (36). The purified rhizospheric and nodule isolates exhibiting high Zn-solubilising potential were designated as rhizospheric ZnSB (Rh-ZnSB) and nodule-associated ZnSB (Nd-ZnSB), respectively and preserved in 70 % (v/v) glycerol at  $-20^\circ\text{C}$  for further characterisation and experimentation.

### Screening for other plant growth-promoting rhizobacterial traits of rhizospheric zinc-solubilising bacteria and nodule-associated zinc-solubilising bacteria

#### Phosphate solubilisation

The Rh-ZnSB and Nd-ZnSB ZnSB isolates were qualitatively screened for phosphate solubilisation using Pikovskaya's agar medium supplemented with tricalcium phosphate [ $\text{Ca}_3(\text{PO}_4)_2$ ] as an insoluble phosphate source. The development of a clear halo zone surrounding bacterial colonies after 72 hr of incubation at  $30 \pm 2^\circ\text{C}$  was considered indicative of phosphate solubilisation activity. The phosphate solubilisation index (P-SI) and phosphate solubilisation efficiency (P-SE) were calculated based on the diameters of the colony and the halo zone, following the method described by previous researchers (37). The results are presented in (Table 2).

For quantitative estimation of phosphate solubilisation, the Rh-ZnSB and Nd-ZnSB isolates were grown in Pikovskaya's broth supplemented with tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ) as the sole phosphorus source. The cultures were incubated at  $30 \pm 2^\circ\text{C}$  under shaking conditions (180 rpm) for 3, 6 and 9 days. At each incubation interval, the cultures were centrifuged and the soluble phosphate content in the supernatant was quantified using the ascorbic acid method described by Murphy and Riley (38). Briefly, the supernatant reacted with ammonium molybdate and ascorbic acid reagents and the absorbance was measured at 600 nm using a spectrophotometer. The concentration of available phosphorus was determined from the supernatant using a calibration curve prepared with tricalcium phosphate and expressed as  $\mu\text{g mg}^{-1}$  (Table 2).

#### Potassium solubilisation

The Rh-ZnSB and Nd-ZnSB isolates were tested for potassium solubilisation using Aleksandrov agar medium containing potassium alumino silicate as the insoluble K source. A clear halo zone around colonies after 72 hr at  $30^\circ\text{C}$  incubation indicated solubilisation of K. The activity was expressed as potassium solubilisation index (K-SI) and potassium efficiency (K-SE) following (39) (Table 3).

Quantitative estimation was carried out in Alexandrov broth, with measurements taken on the 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> days. The Rh- ZnSB and Nd- ZnSB isolates were centrifuged at 12000 rpm for 15 min and potassium content in the supernatant was determined using a flame photometer and with standard curve of KCl. The quantity of solubilisation was expressed in  $\mu\text{g/mL}$  as per J (40) (Table 3).

#### Siderophore production

The Rh-ZnSB and Nd-ZnSB isolates were tested for their ability to solubilise iron using CAS agar medium containing  $\text{FeCl}_3$  and  $\text{FeSO}_4$

as iron sources (41). Pure cultures of the isolates were spot-inoculated on the CAS agar medium and incubated at  $30^\circ\text{C}$  for 7–10 days. The development of yellow to light-orange halos around the colonies indicated siderophore production. The siderophore production index (Fe-SI) and siderophore efficiency (Fe-SE) were calculated using standard formulas as described previously (Table 4).

The quantitative estimation of iron solubilisation by bacterial isolates was done on an iron-deficient succinate medium. The cultures were incubated for 48 hr with continuous agitation at 120 rpm. The siderophore production from each isolate was quantified using the CAS assay described by earlier researchers (41). In this respect, 60.5 mg of CAS was dissolved in 50 mL of deionised water, to which 10 mL of ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) solution was added. In addition, 72.9 mg of hexadecyltrimethylammonium bromide (HDTMA) dissolved in 40 mL of deionised water was added to the CAS solution to make a final volume of 100 mL. Mixing 0.5 mL of this CAS solution with an equal volume of culture supernatant and incubation for 5 min did this. This mixture was read on a spectrophotometer at a wavelength of 630 nm to determine the level of siderophore production (Table 5 and 6).

$$\% \text{ Siderophore} = \frac{\text{Ar-As} \times 100}{\text{Ar}}$$

#### Indole-3-acetic acid production

To detect IAA production, 24 hr old Rh-ZnSB and Nd-ZnSB isolates were inoculated into nutrient broth (pH 7.0) supplemented with 1 % L-tryptophan and incubated at  $28 \pm 2^\circ\text{C}$  for one week. After incubation, the cultures were centrifuged at 3000 rpm for 30 min to obtain the cell-free supernatant. About 2 mL of the supernatant from each isolate were transferred into separate test tubes, followed by the addition of 2–3 drops (approximately 100  $\mu\text{L}$ ) of O-phosphoric acid and 4 mL of Salkowski reagent (prepared by mixing 50 mL of 35 % perchloric acid with 1 mL of 0.5 M  $\text{FeCl}_3$  solution). The reaction mixtures were incubated in the dark for 30 min. The development of a pink color indicated IAA production by the bacterial isolates and absorbance was recorded at 530 nm using a UV-visible spectrophotometer (Table 7) (42).

#### Gibberellic acid production

The Rh-ZnSB and Nd-ZnSB isolates were inoculated into nutrient broth (NB) and incubated at  $28^\circ\text{C}$  for 48 hr. The bacterial cultures were centrifuged at 10000 rpm for 10–15 min to obtain the cell-free supernatant. For  $\text{GA}_3$  estimation, an equal volume of the cell-free extract and ethyl acetate was transferred into a test tube and shaken vigorously for 10 min. The ethyl acetate layer was collected in a separate tube and the extraction was repeated three times. The pooled ethyl acetate fraction was allowed to evaporate at room temperature and the residue was dissolved in absolute alcohol (43). About 2 mL of this extract were mixed with 1 mL of DNPH reagent and incubated at  $100^\circ\text{C}$  for 5 min, followed by cooling in a water bath. Subsequently, 5 mL of 10 % potassium hydroxide (KOH) was added and the mixture was allowed to stand until a red wine colour developed. Then, 15 mL of sterile distilled water was added and the final volume was adjusted to a 1 : 2 dilution using sterile distilled water. The absorbance was measured at 430 nm using a spectrophotometer. The  $\text{GA}_3$  concentration was determined using a standard curve and results were expressed as  $\mu\text{g mL}^{-1}$  (Table 7) (44).

#### Ammonia production

Ammonia production by the Rh-ZnSB and Nd-ZnSB isolates was tested using peptone water. One-day-old bacterial cultures were

inoculated into 10 mL of peptone water in test tubes and incubated for 72 hr at  $36 \pm 1^\circ\text{C}$ . After incubation, Nessler's reagent was added to each tube and the development of a yellow to brown colour was observed, indicating ammonia production (Table 7) (45).

### Consortia development

#### Compatibility test between rhizospheric zinc-solubilising bacteria and nodule-associated zinc-solubilising bacteria

Based on Zn solubilisation ability and other plant growth-promoting traits, the selected nodule isolates (Nd-ZnSB 1, Nd-ZnSB 2, Nd-ZnSB 3) and rhizospheric isolates (Rh-ZnSB 6, Rh-ZnSB 12, Rh-ZnSB 38, Rh-ZnSB 55, Rh-ZnSB 60, Rh-ZnSB 76, Rh-ZnSB 80, Rh-ZnSB 88, Rh-ZnSB 90) were evaluated for compatibility using the cross-inoculation plate assay (Table 8) (46).

#### Preparation of microbial consortia

Selected Nd-ZnSB 1 and three Rh-ZnSB 38, Rh-ZnSB 60, Rh-ZnSB 90 isolates were grown in 100 mL of sterilised YEMB and NB respectively. The each inoculated broth cultures were incubated at  $28 \pm 2^\circ\text{C}$  until they attained the growth level of optical density (OD) = 1 or equivalent to the cell count of  $10^9$ . After attaining the desired growth level, equal volumes from each culture were aseptically transferred into a sterile flask to prepare the microbial consortium prior to use for seed inoculation, soil application and foliar spray (47).

#### Evaluation of rhizospheric zinc-solubilising bacteria and nodule-associated zinc-solubilising bacteria cultures for growth and vigour of lentil

This study was carried out under *in vitro* conditions using the roll towel method. The IPL 120 lentil seeds were used for the study. The seeds were surface-sterilised with 0.05 % sodium hypochlorite, followed by 3–4 rinses with sterile water. The surface-sterilised seeds were soaked in bacterial suspensions ( $10^9$  cells  $\text{mL}^{-1}$ ) for 1 hr. In the paper towel method, the treated seeds were placed on tissue paper while maintaining an appropriate distance between them. The paper was folded properly on both sides and water was sprayed to maintain moisture content. The rolled papers were kept in a beaker containing Jensen's plant nutrient solution and placed on a laboratory bench near sunlight for 15 days. Simultaneously, control plants were maintained by soaking the seeds in sterile water before germination. After 20 days, seedling length was measured and the seed vigour index (SVI) was calculated (48) (Table 9).

$\text{SVI} = \text{Seed germination \%} \times \text{Seedling length (Shoot length + Root length)}$

#### Microcosm experiment

A pot experiment was conducted at the Department of Microbiology, Dr. Rajendra Prasad Central Agricultural University (RPCAU), Pusa, Bihar, (2023) to evaluate the effect of ZnSB isolates and their consortia on lentil growth and Zn availability under reduced fertiliser application. The experiment consisted of eight treatments: T<sub>1</sub>-Control; T<sub>2</sub>- Nd ZnSB (50 % nitrogen-phosphorus-potassium (NPK) + 50 % Zn); T<sub>3</sub> - ZnSB 18 (50 % NPK + Zn); T<sub>4</sub> - ZnSB 20 (50 % NPK + Zn); T<sub>5</sub> - ZnSB 82 (50 % NPK + Zn); T<sub>6</sub> - Zn consortia (50 % NPK + Zn); T<sub>7</sub>- Nd ZnSB 1 + Zn consortia (50 % NPK + Zn); and T<sub>8</sub> - RDF

Nd- ZnSB 1, Rh- ZnSB 38, Rh- ZnSB 60 and Rh- ZnSB 90 were selected based on Zn solubilisation, other plant growth promoting activities and compatibility assay as being better combination for microbial consortia formulation. IPL 120 variety of lentil was used.

Inoculum was prepared by amending concerned broth (50 mL) and incubating at  $30^\circ\text{C}$ , 160 rpm for 24 hr, to maintain 109 colony-forming units (CFU)  $\text{mL}^{-1}$ . Active broth blended with charcoal and lentil seeds were mixed in blended charcoal solution. The seeds used for the RDF treatments (Un-inoculated control) were treated with blank concerned broth and charcoal. Recommended doses of fertilisers (RDF) were used at 20 kg N  $\text{ha}^{-1}$ , 40 kg P  $\text{ha}^{-1}$  and 20 kg K  $\text{ha}^{-1}$  (49). The experimental soil was slightly alkaline in reaction (pH 7.9) and non-saline (EC 0.28  $\text{dS m}^{-1}$ ). The soil contained low organic carbon (0.42 %) and available nitrogen (220 kg  $\text{ha}^{-1}$ ), medium available phosphorus (20.5 kg  $\text{ha}^{-1}$ ) and potassium (155.3 kg  $\text{ha}^{-1}$ ) and was deficient in available Zn (0.42 mg  $\text{kg}^{-1}$ ) and iron (3.2 mg  $\text{kg}^{-1}$ ).

The pot experiment was conducted using a completely randomised design (CRD) with lentil seeds inoculated under eight treatments, arranged randomly and maintained in triplicate. Observations were recorded at two growth stages: 45 days after sowing (DAS), corresponding to the flower initiation stage and 90 DAS, corresponding to the post-flowering stage. At each stage, three plants were randomly uprooted from each treatment to assess plant height (cm), number of branches per plant ( $\text{plant}^{-1}$ ), number of nodules per plant and nodule fresh and dry weight. At the harvesting stage, yield parameters such as number of pods per plant ( $\text{plant}^{-1}$ ), weight of 100 seeds (g) and seed yield (kg  $\text{ha}^{-1}$ ) were recorded. After physiological maturity, the plants were harvested, sun-dried and pods were manually separated. All measurements were recorded in triplicate including soil physiochemical parameters. The Zn and iron (Fe) content in harvested lentil seeds was estimated following di-acid digestion using  $\text{HNO}_3$  and  $\text{HClO}_4$  in a 4 : 1 ratio and quantification was performed using an Atomic Absorption Spectrophotometer (AAS) following the procedure of earlier reports (50).

### Statistical analysis

All statistical analyses for the pot experiment were performed using the mean values of 3 replications for each treatment. The data were subjected to one-way analysis of variance (ANOVA) using Minitab 17 statistical software. Mean separation and comparison among treatments were conducted using Fisher's least significant difference (LSD) test at a 95 % confidence level ( $P \leq 0.05$ ).

## Results and Discussion

### Sampling and isolation of rhizospheric bacterial isolate and nodule isolate

In this present study, rhizospheric soil and root samples were collected from different research fields of the RPCAU farm at Pusa and Dholi ( $25.97$ – $25.99^\circ\text{N}$  and  $85.67$ – $85.68^\circ\text{E}$  respectively). These diverse sampling sites represent a wide range of lentil-growing rhizospheric environments across the RPCAU research farms. A total of 120 rhizospheric soil with 62 root samples were collected. The rhizospheric soil bacterial populations ranged  $0.1 \times 10^3$  to  $1.7 \times 10^3$  CFU  $\text{g}^{-1}$  soil (Supplementary Table 1). Out of the 62 root samples only 18 root samples had healthy nodulation in which bacterial population count was found from  $0.3 \times 10^3$  to  $0.9 \times 10^3$  CFU  $\text{g}^{-1}$  nodule compared to the rhizospheric soil sample. Total 30 morphotypes were obtained from 18 root nodule samples (Supplementary Table 2). A total of 157 bacterial isolates were obtained of soil and root nodules (Supplementary Table 3).

## Screening of isolates for zinc solubilisation in rhizospheric zinc-solubilising bacteria and nodule associated zinc-solubilising bacteria

### Rhizospheric zinc-solubilising bacteria group

In this present study, ZnO and ZnCO<sub>3</sub> sources used, from these sources the most preferred Zn source is ZnO for both Rh- ZnSB and Nd- ZnSB group. Whereas, ZnCO<sub>3</sub> were found to be least favored substrate, among the Rh- ZnSB group of isolates there was considerable difference were measured qualitatively. Out of a total of 127 rhizospheric isolates, 68 showed positive when tested on ZnO-amended medium. The Zn-SE values of ZnO-positive Rh-ZnSB isolates ranged from 25.00 to 416.70, with an average Zn-SE of 110.96, indicating wide variability in Zn solubilisation potential. Among the best-performing isolates, the highest Zn-SE was recorded in Rh-38 (416.70 ± 32.54), followed by Rh-90 (300.00 ± 23.43) and Rh-60 (285.70 ± 22.31). Other promising isolates included Rh-88 (271.40 ± 21.20), Rh-82 (257.10 ± 20.08), Rh-20 (244.40 ± 19.09) and Rh-55 (200.00 ± 15.62), demonstrating strong Zn solubilisation ability on ZnO (Supplementary Table 4 and Fig.1a). Similarly, when ZnCO<sub>3</sub> was used as the Zn source, out of a total of 127 rhizospheric isolates, 76 isolates showed positive Zn solubilisation. The Zn-SE values of ZnCO<sub>3</sub>-positive Rh-isolates ranged from 20.00 to 271.43, with an average Zn-SE of 92.40. The highest Zn-SE on ZnCO<sub>3</sub> was recorded in Rh-38 (271.43 ± 21.20), followed by Rh-90 (220.00 ± 17.18), Rh-6 (200.00 ± 8.72), Rh-88 (175.00 ± 13.67) and Rh-70 (166.67 ± 13.02) (Supplementary Table 5).

The quantitative Zn solubilisation of Rh-ZnSB isolates on ZnO and ZnCO<sub>3</sub> were also performed upto 11<sup>th</sup> day with interval of 2 days (Table 1). On the ZnO medium, Zn solubilisation (ppm) on the 9<sup>th</sup> day was ranged from 2.51 ± 0.20 to 3.93 ± 0.12 with highest activity was observed in Rh-ZnSB 38 (3.93 ± 0.12), Rh-ZnSB 60 (3.79 ± 0.17) and Rh-ZnSB 76 (3.78 ± 0.16), which were statistically at par. Similarly on ZnCO<sub>3</sub> medium, 9<sup>th</sup> day was ranged from 1.92 ± 0.15 (Rh-ZnSB 80) to 3.51 ± 0.11 ppm (Rh-ZnSB 38) with maximum was recorded in Rh-ZnSB 38 (3.51 ± 0.11) followed by Rh-ZnSB 60 and Rh-ZnSB 76 (3.50 ± 0.15 each) (Table 1).

### Nodule associated zinc-solubilising bacteria group

Among the nodule isolates, 23 isolates indicated positive when tested with ZnO, the range of Zn-SE isolates ranged from 57.10 to

357.10 with an average Zn-SE of 113.92. The maximum Zn-SE was recorded in Nd-isolate 1 (357.10 ± 27.89), followed by Nd-isolate 2 (342.90 ± 26.78) and Nd-isolate 3 (300.00 ± 23.43) (Supplementary Table 4). Similarly, out of 30 Nd-isolates, 24 isolates exhibited positive when tested with ZnCO<sub>3</sub> source, the Zn-SE values of Nd-ZnSB isolates ranged from 33.33 to 200.00, with an average Zn-SE of approximately 86.6. The maximum Zn-SE was recorded in Nd-isolate 1 (200.00 ± 15.62), followed by Nd-isolate 2 (160.00 ± 12.50) and Nd-isolate 3 (150.00 ± 11.72) (Supplementary Table 5).

The Zn quantification of Nd-ZnSB isolates on ZnO and ZnCO<sub>3</sub> were also performed up to the 11<sup>th</sup> day with interval of 2 days (Table 1). Under ZnO source, at 9<sup>th</sup> DAI, the Zn quantification was ranged from 2.40 to 3.77, with the highest solubilisation recorded in Nd-ZnSB 1 (3.77 ± 0.29 ppm), followed by Nd-ZnSB 2 (2.72 ± 0.21 ppm). Similarly, under ZnCO<sub>3</sub> source, at 9 DAI Zn solubilisation (ppm) was recorded from 1.26 to 3.28 ppm, with the maximum solubilisation were observed in Nd-ZnSB 1 (3.28 ± 0.26 ppm), followed by Nd-ZnSB 2 (2.41 ± 0.19) (Table 1).

### Screening of the isolates for plant growth-promoting traits

All selected isolates exhibited multiple plant growth-promoting traits, including phosphate and potassium solubilisation, siderophore production, indole-3-acetic acid (IAA), gibberellic acid (GA<sub>3</sub>), ammonia production.

### Phosphate solubilisation

The qualitative evaluation of phosphate solubilisation efficiency (PSE %) of (Rh- ZnSB) isolates showed wide variation, ranging from (300.00 ± 13.08 % to 518.33 ± 40.48). Among all the isolates, Rh-ZnSB 90 exhibited the highest phosphate solubilisation efficiency (518.33 ± 40.48), closely followed by Rh-ZnSB 88 (516.67 ± 40.35) and Rh-ZnSB 60 (457.14 ± 19.93) (Table 2 and Fig.1b). Among the Nd-ZnSB isolates, phosphate solubilisation efficiency (PSE %) ranged from (342.86 ± 26.78 to 503.51 ± 39.33). The highest P-solubilisation efficiency was recorded in Nd-ZnSB 1 (503.51 ± 39.33 %), followed by Nd- ZnSB 3 (357.14 ± 27.89) (Fig.1b, Table 2)

At the 7<sup>th</sup> day after inoculation, the phosphorus quantification among the rhizospheric Fe-solubilising bacterial isolates ranged from 0.81 ± 0.06 to 2.02 ± 0.09 mg mL<sup>-1</sup>. The highest phosphorus solubilisation was recorded in Rh-ZnSB 60 (2.02 ± 0.09 mg mL<sup>-1</sup>), followed by Rh-ZnSB 76 (1.91 ± 0.08 mg mL<sup>-1</sup>) and Rh-ZnSB 38 (1.84 ±

**Table 1.** Quantitative estimation of zinc solubilisation activity of bacterial isolates and rhizobium at different time intervals with two different zinc sources

|            | ZnO (ppm)                 |                           |                            |                           |                           | ZnCO <sub>3</sub> (ppm)   |                           |                           |                           |                           |
|------------|---------------------------|---------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|            | 3DAI                      | 5 DAI                     | 7 DAI                      | 9 DAI                     | 11 DAI                    | 3DAI                      | 5 DAI                     | 7 DAI                     | 9 DAI                     | 11 DAI                    |
| Rh-ZnSB 6  | 0.82 ± 0.05 <sup>f</sup>  | 1.73 ± 0.11 <sup>e</sup>  | 2.43 ± 0.15 <sup>e</sup>   | 3.73 ± 0.23 <sup>ab</sup> | 1.12 ± 0.07 <sup>d</sup>  | 1.93 ± 0.12 <sup>bc</sup> | 0.70 ± 0.04 <sup>h</sup>  | 2.15 ± 0.13 <sup>de</sup> | 2.41 ± 0.15 <sup>ef</sup> | 1.85 ± 0.12 <sup>e</sup>  |
| Rh-ZnSB 12 | 1.00 ± 0.05 <sup>e</sup>  | 1.82 ± 0.09 <sup>e</sup>  | 2.52 ± 0.13 <sup>de</sup>  | 2.64 ± 0.13 <sup>c</sup>  | 0.92 ± 0.05 <sup>e</sup>  | 2.13 ± 0.11 <sup>b</sup>  | 2.10 ± 0.11 <sup>e</sup>  | 2.32 ± 0.12 <sup>d</sup>  | 2.53 ± 0.13 <sup>ef</sup> | 1.72 ± 0.09 <sup>ef</sup> |
| Rh-ZnSB 20 | 0.78 ± 0.05 <sup>f</sup>  | 2.12 ± 0.13 <sup>d</sup>  | 2.65 ± 0.16 <sup>cde</sup> | 2.71 ± 0.16 <sup>c</sup>  | 0.98 ± 0.06 <sup>de</sup> | 1.67 ± 0.10 <sup>d</sup>  | 2.11 ± 0.13 <sup>e</sup>  | 2.40 ± 0.15 <sup>cd</sup> | 2.71 ± 0.17 <sup>de</sup> | 2.10 ± 0.13 <sup>d</sup>  |
| Rh-ZnSB 38 | 2.71 ± 0.08 <sup>a</sup>  | 2.89 ± 0.09 <sup>a</sup>  | 3.76 ± 0.11 <sup>a</sup>   | 3.93 ± 0.12 <sup>a</sup>  | 2.30 ± 0.07 <sup>a</sup>  | 2.55 ± 0.08 <sup>a</sup>  | 2.81 ± 0.08 <sup>a</sup>  | 3.29 ± 0.10 <sup>a</sup>  | 3.51 ± 0.11 <sup>a</sup>  | 1.63 ± 0.05 <sup>ef</sup> |
| Rh-ZnSB 55 | 0.90 ± 0.04 <sup>ef</sup> | 0.91 ± 0.04 <sup>f</sup>  | 2.87 ± 0.13 <sup>c</sup>   | 3.64 ± 0.16 <sup>ab</sup> | 0.92 ± 0.04 <sup>e</sup>  | 1.68 ± 0.07 <sup>d</sup>  | 2.31 ± 0.10 <sup>de</sup> | 2.70 ± 0.12 <sup>b</sup>  | 2.99 ± 0.13 <sup>cd</sup> | 2.34 ± 0.10 <sup>cd</sup> |
| Rh-ZnSB 60 | 2.31 ± 0.10 <sup>b</sup>  | 2.67 ± 0.12 <sup>b</sup>  | 3.65 ± 0.16 <sup>ab</sup>  | 3.79 ± 0.17 <sup>ab</sup> | 2.10 ± 0.09 <sup>b</sup>  | 2.45 ± 0.11 <sup>a</sup>  | 2.75 ± 0.12 <sup>ab</sup> | 3.29 ± 0.14 <sup>a</sup>  | 3.50 ± 0.15 <sup>ab</sup> | 2.62 ± 0.11 <sup>b</sup>  |
| Rh-ZnSB 76 | 2.30 ± 0.10 <sup>b</sup>  | 2.66 ± 0.12 <sup>b</sup>  | 3.63 ± 0.16 <sup>ab</sup>  | 3.78 ± 0.16 <sup>ab</sup> | 2.10 ± 0.09 <sup>b</sup>  | 2.45 ± 0.11 <sup>a</sup>  | 2.41 ± 0.11 <sup>cd</sup> | 3.29 ± 0.14 <sup>a</sup>  | 3.50 ± 0.15 <sup>ab</sup> | 2.56 ± 0.11 <sup>bc</sup> |
| Rh-ZnSB 80 | 0.80 ± 0.06 <sup>f</sup>  | 0.86 ± 0.07 <sup>f</sup>  | 1.79 ± 0.14 <sup>f</sup>   | 2.51 ± 0.20 <sup>c</sup>  | 0.98 ± 0.08 <sup>de</sup> | 1.90 ± 0.15 <sup>c</sup>  | 1.61 ± 0.13 <sup>f</sup>  | 1.92 ± 0.15 <sup>e</sup>  | 2.26 ± 0.18 <sup>g</sup>  | 1.72 ± 0.13 <sup>ef</sup> |
| Rh-ZnSB 88 | 0.90 ± 0.07 <sup>ef</sup> | 2.32 ± 0.18 <sup>cd</sup> | 3.45 ± 0.27 <sup>b</sup>   | 3.51 ± 0.27 <sup>b</sup>  | 0.86 ± 0.07 <sup>e</sup>  | 2.13 ± 0.17 <sup>b</sup>  | 2.34 ± 0.18 <sup>cd</sup> | 2.67 ± 0.21 <sup>bc</sup> | 3.00 ± 0.23 <sup>cd</sup> | 2.33 ± 0.18 <sup>cd</sup> |
| Rh-ZnSB 90 | 1.70 ± 0.13 <sup>d</sup>  | 2.33 ± 0.18 <sup>c</sup>  | 2.89 ± 0.23 <sup>c</sup>   | 3.72 ± 0.29 <sup>ab</sup> | 1.62 ± 0.13 <sup>c</sup>  | 2.13 ± 0.17 <sup>b</sup>  | 2.56 ± 0.20 <sup>bc</sup> | 2.88 ± 0.23 <sup>b</sup>  | 3.20 ± 0.25 <sup>bc</sup> | 2.56 ± 0.20 <sup>bc</sup> |
| Nd-ZnSB 1  | 2.30 ± 0.18 <sup>b</sup>  | 2.60 ± 0.20 <sup>b</sup>  | 2.81 ± 0.22 <sup>cd</sup>  | 3.77 ± 0.29 <sup>ab</sup> | 2.11 ± 0.16 <sup>b</sup>  | 2.45 ± 0.19 <sup>a</sup>  | 2.47 ± 0.19 <sup>cd</sup> | 3.28 ± 0.26 <sup>a</sup>  | 3.47 ± 0.27 <sup>ab</sup> | 2.92 ± 0.23 <sup>a</sup>  |
| Nd-ZnSB 2  | 2.00 ± 0.16 <sup>c</sup>  | 0.78 ± 0.06 <sup>f</sup>  | 2.51 ± 0.20 <sup>e</sup>   | 2.72 ± 0.21 <sup>c</sup>  | 2.38 ± 0.19 <sup>a</sup>  | 1.91 ± 0.15 <sup>c</sup>  | 2.10 ± 0.16 <sup>e</sup>  | 2.41 ± 0.19 <sup>cd</sup> | 2.65 ± 0.21 <sup>e</sup>  | 2.41 ± 0.19 <sup>bc</sup> |
| Nd-ZnSB 3  | 2.00 ± 0.16 <sup>c</sup>  | 0.77 ± 0.06 <sup>f</sup>  | 1.98 ± 0.15 <sup>f</sup>   | 2.40 ± 0.19 <sup>c</sup>  | 2.32 ± 0.18 <sup>a</sup>  | 0.86 ± 0.07 <sup>e</sup>  | 0.95 ± 0.07 <sup>g</sup>  | 1.26 ± 0.10 <sup>f</sup>  | 1.99 ± 0.16 <sup>g</sup>  | 1.50 ± 0.12 <sup>f</sup>  |
| CD         | 0.18                      | 0.2                       | 0.29                       | 0.35                      | 0.18                      | 0.21                      | 0.22                      | 0.27                      | 0.31                      | 0.24                      |
| CV (%)     | 6.65                      | 6.45                      | 6.15                       | 6.28                      | 6.85                      | 6.31                      | 6.36                      | 6.25                      | 6.26                      | 6.6                       |

DAI: days after inoculation; ZnO: zinc oxide; ZnCO<sub>3</sub>: zinc carbonate; Rh-ZnSB: rhizosphere ZnSB isolate; Nd-ZnSB: nodule-derived ZnSB isolate; CD: critical difference ( $p \leq 0.05$ ); CV: coefficient of variation.

**Table 2.** Evaluation of efficient bacterial isolates of qualitative and quantitative assay on phosphorus solubilisation

| Bacterial  | P Qualitative              |                          |                           |                              | P Quantitative           |                           |                           |                           |
|------------|----------------------------|--------------------------|---------------------------|------------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
|            | SD                         | CD                       | PSI                       | PSE (%)                      | 3DAI                     | 5DAI                      | 7DAI                      | 9DAI                      |
| Rh-ZnSB 6  | -                          | -                        | -                         | -                            | -                        | -                         | -                         | -                         |
| Rh-ZnSB 12 | 21.50 ± 1.07 <sup>bc</sup> | 7.00 ± 0.35 <sup>b</sup> | 4.07 ± 0.20 <sup>c</sup>  | 307.14 ± 15.36 <sup>de</sup> | 0.33 ± 0.02 <sup>e</sup> | 0.61 ± 0.03 <sup>ef</sup> | 1.51 ± 0.08 <sup>d</sup>  | 1.31 ± 0.07 <sup>d</sup>  |
| Rh-ZnSB 20 | 15.00 ± 0.91 <sup>f</sup>  | 6.00 ± 0.36 <sup>c</sup> | 3.50 ± 0.21 <sup>d</sup>  | 350.00 ± 21.29 <sup>cd</sup> | 0.34 ± 0.02 <sup>e</sup> | 0.65 ± 0.04 <sup>de</sup> | 1.52 ± 0.09 <sup>d</sup>  | 1.32 ± 0.08 <sup>d</sup>  |
| Rh-ZnSB 38 | 17.00 ± 0.51 <sup>ef</sup> | 6.00 ± 0.18 <sup>c</sup> | 3.83 ± 0.11 <sup>cd</sup> | 383.33 ± 11.50 <sup>c</sup>  | 0.56 ± 0.02 <sup>c</sup> | 1.50 ± 0.04 <sup>b</sup>  | 1.84 ± 0.06 <sup>c</sup>  | 1.64 ± 0.05 <sup>c</sup>  |
| Rh-ZnSB 55 | 20.00 ± 0.87 <sup>cd</sup> | 7.00 ± 0.31 <sup>b</sup> | 3.86 ± 0.17 <sup>cd</sup> | 385.71 ± 16.81 <sup>c</sup>  | 0.46 ± 0.02 <sup>d</sup> | 0.70 ± 0.03 <sup>de</sup> | 1.50 ± 0.07 <sup>d</sup>  | 1.30 ± 0.06 <sup>d</sup>  |
| Rh-ZnSB 60 | 25.00 ± 1.09 <sup>a</sup>  | 7.00 ± 0.31 <sup>b</sup> | 4.57 ± 0.20 <sup>b</sup>  | 457.14 ± 19.93 <sup>b</sup>  | 0.56 ± 0.02 <sup>c</sup> | 1.52 ± 0.07 <sup>b</sup>  | 2.02 ± 0.09 <sup>b</sup>  | 1.82 ± 0.08 <sup>b</sup>  |
| Rh-ZnSB 76 | 18.00 ± 0.78 <sup>de</sup> | 9.00 ± 0.39 <sup>a</sup> | 3.00 ± 0.13 <sup>e</sup>  | 300.00 ± 13.08 <sup>e</sup>  | 0.56 ± 0.02 <sup>c</sup> | 1.51 ± 0.07 <sup>b</sup>  | 1.91 ± 0.08 <sup>bc</sup> | 1.71 ± 0.07 <sup>bc</sup> |
| Rh-ZnSB 80 | 19.00 ± 1.48 <sup>de</sup> | 7.00 ± 0.55 <sup>b</sup> | 3.71 ± 0.29 <sup>cd</sup> | 371.43 ± 29.01 <sup>c</sup>  | 0.21 ± 0.02 <sup>f</sup> | 0.50 ± 0.04 <sup>f</sup>  | 0.81 ± 0.06 <sup>e</sup>  | 0.61 ± 0.05 <sup>f</sup>  |
| Rh-ZnSB 88 | 25.00 ± 1.95 <sup>a</sup>  | 6.00 ± 0.47 <sup>c</sup> | 5.17 ± 0.40 <sup>a</sup>  | 516.67 ± 40.35 <sup>a</sup>  | 0.72 ± 0.06 <sup>b</sup> | 1.31 ± 0.10 <sup>c</sup>  | 1.63 ± 0.13 <sup>d</sup>  | 1.43 ± 0.11 <sup>d</sup>  |
| Rh-ZnSB 90 | 25.10 ± 1.96 <sup>a</sup>  | 6.00 ± 0.47 <sup>c</sup> | 5.18 ± 0.40 <sup>a</sup>  | 518.33 ± 40.48 <sup>a</sup>  | 0.35 ± 0.03 <sup>e</sup> | 0.62 ± 0.05 <sup>ef</sup> | 0.96 ± 0.07 <sup>e</sup>  | 0.76 ± 0.06 <sup>e</sup>  |
| Nd-ZnSB 1  | 23.00 ± 1.80 <sup>ab</sup> | 5.70 ± 0.45 <sup>c</sup> | 5.04 ± 0.39 <sup>a</sup>  | 503.51 ± 39.33 <sup>a</sup>  | 0.99 ± 0.08 <sup>a</sup> | 2.52 ± 0.20 <sup>a</sup>  | 2.30 ± 0.18 <sup>a</sup>  | 2.10 ± 0.16 <sup>a</sup>  |
| Nd-ZnSB 2  | 17.00 ± 1.33 <sup>ef</sup> | 7.00 ± 0.55 <sup>b</sup> | 3.43 ± 0.27 <sup>de</sup> | 342.86 ± 26.78 <sup>cd</sup> | 0.45 ± 0.04 <sup>d</sup> | 0.75 ± 0.06 <sup>d</sup>  | 0.91 ± 0.07 <sup>e</sup>  | 0.71 ± 0.06 <sup>ef</sup> |
| Nd-ZnSB 3  | 18.00 ± 1.41 <sup>de</sup> | 7.00 ± 0.55 <sup>b</sup> | 3.57 ± 0.28 <sup>d</sup>  | 357.14 ± 27.89 <sup>c</sup>  | 0.43 ± 0.03 <sup>d</sup> | 0.75 ± 0.06 <sup>d</sup>  | 0.90 ± 0.07 <sup>e</sup>  | 0.70 ± 0.05 <sup>ef</sup> |
| CD         | <b>2.17</b>                | <b>0.68</b>              | <b>0.44</b>               | <b>43.72</b>                 | <b>0.06</b>              | <b>0.13</b>               | <b>0.15</b>               | <b>0.13</b>               |
| CV( %)     | <b>6.88</b>                | <b>6.57</b>              | <b>6.99</b>               | <b>7.07</b>                  | <b>7.44</b>              | <b>7.58</b>               | <b>6.54</b>               | <b>6.59</b>               |

SD: solubilisation diameter; CD: colony diameter; PSI: phosphate solubilisation index; PSE: phosphate solubilisation efficiency; DAI: days after inoculation; Rh-ZnSB: rhizosphere ZnSB isolate; Nd-ZnSB: nodule-derived ZnSB isolate; CD (statistical): critical difference ( $p \leq 0.05$ ); CV: coefficient of variation.

0.06 mg mL<sup>-1</sup>) (Table 2). In the case of Nd-ZnSB, phosphorus quantification ranged from (0.90 ± 0.07 to 2.30 ± 0.18 mg mL<sup>-1</sup>). The highest value was observed in Nd-ZnSB 1 (2.30 ± 0.18 mg mL<sup>-1</sup>), followed by Nd-ZnSB 2 (0.91 ± 0.07 mg mL<sup>-1</sup>) (Table 2).

#### Potassium solubilisation

The potassium solubilisation efficiency of the bacterial isolates varied significantly among the isolates. In the Rh-ZnSB group, (K-SE %) ranged from 210.00 ± 9.15 to 333.30 ± 14.53. The highest KSE were recorded in Rh-ZnSB 60 333.30 ± 14.53, followed by Rh-ZnSB 20 302.00 ± 18.37 and Rh-ZnSB 38 (280.00 ± 8.40 %). In contrast, the Nd-ZnSB group exhibited a comparatively lower range of (K-SE %) varying from (155.60 to 266.70). Among these, Nd-ZnSB 1 (266.70 ± 20.83) showed the highest efficiency, followed by Nd-ZnSB 2 (216.70 ± 16.92) (Fig.1c, Table 3)

At the 7<sup>th</sup> day after inoculation, potassium quantification among rhizospheric Fe-solubilising bacterial isolates ranged from 13.14 ± 0.57 to 19.12 ± 1.49 mg mL<sup>-1</sup>. The highest value was recorded in Rh-ZnSB 88 (19.12 ± 1.49), followed by Rh-ZnSB 12 (18.21 ± 0.91) and Rh-ZnSB 76 (18.10 ± 0.79). Among the Nd-ZnSB isolates, values at 7 DAI ranged from 14.82 ± 1.16 to 19.12 ± 1.49 mg mL<sup>-1</sup>. The maximum value was observed in Nd-ZnSB 1 (19.12 ± 1.49), followed by Nd-ZnSB 2 (14.82 ± 1.16) (Table 3).

#### Siderophore production

Qualitatively siderophore production by bacterial isolates showed

clear differences in two Zn sources i.e., FeCl<sub>3</sub> and FeSO<sub>4</sub>. Under ferric chloride (FC) as the iron source, Rh-ZnSB isolates exhibited FeSE (%) ranged from 240.70 ± 18.80 to 361.10 ± 10.83. The highest FeSE (%) was recorded in Rh-ZnSB 38 (361.10 ± 10.83), followed by Rh-ZnSB 60 (322.70 ± 14.07) and Rh-ZnSB 20 (315.80 ± 19.21 %). When ferrous sulphate (FS) was used as the iron source, Rh-ZnSB isolates showed higher FeSE values, ranging from 304.50 ± 13.27 % to 400.00 ± 12.00 %. The highest FeSE was recorded in Rh-ZnSB 38 (400.00 ± 12.00 %), followed by Rh-ZnSB 12 and Rh-ZnSB 90 (380.00 ± 19.00 % and 380.00 ± 29.68 %, respectively) and Rh-ZnSB 76 (368.40 ± 16.06 %), demonstrating enhanced siderophore efficiency under FS compared to FC (Fig.1d, Table 4).

Among the Nd-ZnSB isolates, FeSE values under FC ranged from 315.80 ± 24.66 % to 325.00 ± 25.38 %. The maximum FeSE was observed in Nd-ZnSB 1 (325.00 ± 25.38 %), followed closely by Nd-ZnSB 2 (315.80 ± 24.66 %), indicating comparable siderophore efficiency under ferric chloride. For the Nd-ZnSB isolates, FeSE under FS ranged from 325.00 ± 25.38 % to 392.90 ± 30.69 %. The maximum efficiency was observed in Nd-ZnSB 1 (392.90 ± 30.69 %), followed by Nd-ZnSB 2 (325.00 ± 25.38 %), indicating superior siderophore-mediated iron mobilisation under FS (Table 4).

Quantitative siderophore production by bacterial isolates showed clear differences in two Zn sources i.e., FeCl<sub>3</sub> and FeSO<sub>4</sub>. At 11 days after incubation (11 DAS). Under ferric chloride (FC) at 11

**Table 3.** Evaluation of efficient bacterial isolates of qualitative and quantitative assay on potassium solubilisation

| Bacterial  | K Qualitative (%)          |                          |                           |                              | K Quantitative (mg mL <sup>-1</sup> ) |                             |                            |                            |
|------------|----------------------------|--------------------------|---------------------------|------------------------------|---------------------------------------|-----------------------------|----------------------------|----------------------------|
|            | SD                         | CD                       | KSI                       | KSE (%)                      | 3DAI                                  | 5DAI                        | 7DAI                       | 9DAI                       |
| Rh-ZnSB 6  | -                          | -                        | -                         | -                            | -                                     | -                           | -                          | -                          |
| Rh-ZnSB 12 | 21.00 ± 1.05 <sup>a</sup>  | 9.00 ± 0.45 <sup>a</sup> | 3.30 ± 0.17 <sup>de</sup> | 233.30 ± 11.67 <sup>ef</sup> | 17.51 ± 0.88 <sup>ab</sup>            | 17.61 ± 0.88 <sup>ab</sup>  | 18.21 ± 0.91 <sup>ab</sup> | 22.51 ± 1.13 <sup>b</sup>  |
| Rh-ZnSB 20 | 15.10 ± 0.92 <sup>de</sup> | 5.00 ± 0.30 <sup>d</sup> | 4.00 ± 0.24 <sup>ab</sup> | 302.00 ± 18.37 <sup>b</sup>  | 15.86 ± 0.96 <sup>cd</sup>            | 15.96 ± 0.97 <sup>cd</sup>  | 16.84 ± 1.02 <sup>b</sup>  | 18.50 ± 1.13 <sup>cd</sup> |
| Rh-ZnSB 38 | 14.00 ± 0.42 <sup>ef</sup> | 5.00 ± 0.15 <sup>d</sup> | 3.80 ± 0.11 <sup>bc</sup> | 280.00 ± 8.40 <sup>bc</sup>  | 12.25 ± 0.37 <sup>e</sup>             | 12.35 ± 0.37 <sup>e</sup>   | 14.40 ± 0.43 <sup>cd</sup> | 15.00 ± 0.45 <sup>e</sup>  |
| Rh-ZnSB 55 | 10.50 ± 0.46 <sup>g</sup>  | 5.00 ± 0.22 <sup>d</sup> | 3.10 ± 0.14 <sup>e</sup>  | 210.00 ± 9.15 <sup>f</sup>   | 12.50 ± 0.54 <sup>e</sup>             | 12.60 ± 0.55 <sup>e</sup>   | 13.14 ± 0.57 <sup>d</sup>  | 14.20 ± 0.62 <sup>e</sup>  |
| Rh-ZnSB 60 | 17.00 ± 0.74 <sup>bc</sup> | 5.10 ± 0.22 <sup>d</sup> | 4.30 ± 0.19 <sup>a</sup>  | 333.30 ± 14.53 <sup>a</sup>  | 15.40 ± 0.67 <sup>d</sup>             | 15.50 ± 0.68 <sup>d</sup>   | 17.18 ± 0.75 <sup>b</sup>  | 18.10 ± 0.79 <sup>d</sup>  |
| Rh-ZnSB 76 | 15.00 ± 0.65 <sup>de</sup> | 6.00 ± 0.26 <sup>c</sup> | 3.50 ± 0.15 <sup>cd</sup> | 250.00 ± 10.90 <sup>de</sup> | 16.30 ± 0.71 <sup>bcd</sup>           | 16.40 ± 0.71 <sup>bcd</sup> | 18.10 ± 0.79 <sup>ab</sup> | 19.20 ± 0.84 <sup>cd</sup> |
| Rh-ZnSB 80 | 0.00 ± 0.00 <sup>h</sup>   | 0.00 ± 0.00 <sup>e</sup> | 0.00 ± 0.00 <sup>g</sup>  | 0.00 ± 0.00 <sup>i</sup>     | 0.00 ± 0.00 <sup>g</sup>              | 0.00 ± 0.00 <sup>g</sup>    | 0.00 ± 0.00 <sup>e</sup>   | 0.00 ± 0.00 <sup>f</sup>   |
| Rh-ZnSB 88 | 20.00 ± 1.56 <sup>a</sup>  | 9.00 ± 0.70 <sup>a</sup> | 3.20 ± 0.25 <sup>de</sup> | 222.20 ± 17.35 <sup>fg</sup> | 18.45 ± 1.44 <sup>a</sup>             | 18.55 ± 1.45 <sup>a</sup>   | 19.12 ± 1.49 <sup>a</sup>  | 20.15 ± 1.57 <sup>c</sup>  |
| Rh-ZnSB 90 | 18.00 ± 1.41 <sup>b</sup>  | 8.10 ± 0.63 <sup>b</sup> | 3.20 ± 0.25 <sup>de</sup> | 222.20 ± 17.35 <sup>fg</sup> | 17.20 ± 1.34 <sup>abc</sup>           | 17.30 ± 1.35 <sup>abc</sup> | 17.25 ± 1.35 <sup>b</sup>  | 25.61 ± 2.00 <sup>a</sup>  |
| Nd-ZnSB 1  | 16.00 ± 1.25 <sup>cd</sup> | 6.00 ± 0.47 <sup>c</sup> | 3.70 ± 0.29 <sup>bc</sup> | 266.70 ± 20.83 <sup>cd</sup> | 18.55 ± 1.45 <sup>a</sup>             | 18.65 ± 1.46 <sup>a</sup>   | 19.12 ± 1.49 <sup>a</sup>  | 23.21 ± 1.81 <sup>b</sup>  |
| Nd-ZnSB 2  | 13.00 ± 1.02 <sup>f</sup>  | 6.00 ± 0.47 <sup>c</sup> | 3.20 ± 0.25 <sup>de</sup> | 216.70 ± 16.92 <sup>fg</sup> | 1.90 ± 0.15 <sup>f</sup>              | 2.00 ± 0.16 <sup>f</sup>    | 13.50 ± 1.05 <sup>cd</sup> | 14.81 ± 1.16 <sup>e</sup>  |
| Nd-ZnSB 3  | 14.00 ± 1.09 <sup>ef</sup> | 9.00 ± 0.70 <sup>a</sup> | 2.60 ± 0.20 <sup>f</sup>  | 155.60 ± 12.15 <sup>h</sup>  | 13.55 ± 1.06 <sup>e</sup>             | 13.65 ± 1.07 <sup>e</sup>   | 14.82 ± 1.16 <sup>c</sup>  | 15.10 ± 1.18 <sup>e</sup>  |
| CD         | 1.58                       | 0.71                     | 0.33                      | 22.94                        | 1.49                                  | 1.5                         | 1.63                       | 1.92                       |
| CV( %)     | 7.05                       | 7.49                     | 6.65                      | 6.60                         | 7.22                                  | 7.22                        | 6.96                       | 7.19                       |

**Table 4.** Characterisation of efficient zinc solubilising bacterial isolates on Siderophore Production at two different iron sources of ferrous sulphate and ferric chloride

|            | FC-siderophore production  |                            |                            |                               | FS- siderophore production |                            |                             |                               |
|------------|----------------------------|----------------------------|----------------------------|-------------------------------|----------------------------|----------------------------|-----------------------------|-------------------------------|
|            | CD                         | SD                         | FeSI                       | FeSE                          | CD                         | SD                         | FeSI                        | FeSE                          |
| Rh-ZnSB 6  | 17.00 ± 1.06 <sup>e</sup>  | 50.00 ± 3.12 <sup>c</sup>  | 3.94 ± 0.25 <sup>bcd</sup> | 294.10 ± 18.37 <sup>cde</sup> | 19.00 ± 1.19 <sup>c</sup>  | 60.00 ± 3.75 <sup>bc</sup> | 4.20 ± 0.26 <sup>ef</sup>   | 315.80 ± 19.72 <sup>de</sup>  |
| Rh-ZnSB 12 | 18.00 ± 0.90 <sup>de</sup> | 50.00 ± 2.50 <sup>c</sup>  | 3.78 ± 0.19 <sup>cde</sup> | 277.80 ± 13.89 <sup>de</sup>  | 15.00 ± 0.75 <sup>e</sup>  | 57.00 ± 2.85 <sup>c</sup>  | 4.80 ± 0.24 <sup>abc</sup>  | 380.00 ± 19.00 <sup>ab</sup>  |
| Rh-ZnSB 20 | 19.00 ± 1.16 <sup>de</sup> | 60.00 ± 3.65 <sup>b</sup>  | 4.16 ± 0.25 <sup>bc</sup>  | 315.80 ± 19.21 <sup>bc</sup>  | 21.00 ± 1.28 <sup>ab</sup> | 68.00 ± 4.14 <sup>a</sup>  | 4.20 ± 0.26 <sup>ef</sup>   | 323.80 ± 19.70 <sup>ab</sup>  |
| Rh-ZnSB 38 | 18.00 ± 0.54 <sup>de</sup> | 65.00 ± 1.95 <sup>ab</sup> | 4.61 ± 0.14 <sup>a</sup>   | 361.10 ± 10.83 <sup>a</sup>   | 15.00 ± 0.45 <sup>e</sup>  | 60.00 ± 1.80 <sup>bc</sup> | 5.00 ± 0.15 <sup>a</sup>    | 400.00 ± 12.00 <sup>a</sup>   |
| Rh-ZnSB 55 | 20.00 ± 0.87 <sup>cd</sup> | 60.00 ± 2.62 <sup>b</sup>  | 4.00 ± 0.17 <sup>bcd</sup> | 300.00 ± 13.08 <sup>bcd</sup> | 22.00 ± 0.96 <sup>a</sup>  | 67.00 ± 2.92 <sup>a</sup>  | 4.00 ± 0.17 <sup>f</sup>    | 304.50 ± 13.27 <sup>e</sup>   |
| Rh-ZnSB 60 | 22.00 ± 0.96 <sup>c</sup>  | 71.00 ± 3.09 <sup>a</sup>  | 4.23 ± 0.18 <sup>ab</sup>  | 322.70 ± 14.07 <sup>bc</sup>  | 20.00 ± 0.87 <sup>bc</sup> | 68.00 ± 2.96 <sup>a</sup>  | 4.40 ± 0.19 <sup>cdef</sup> | 340.00 ± 14.82 <sup>cde</sup> |
| Rh-ZnSB 76 | 25.00 ± 1.09 <sup>b</sup>  | 68.00 ± 2.96 <sup>a</sup>  | 3.72 ± 0.16 <sup>de</sup>  | 272.00 ± 11.86 <sup>def</sup> | 19.00 ± 0.83 <sup>c</sup>  | 70.00 ± 3.05 <sup>a</sup>  | 4.70 ± 0.20 <sup>abcd</sup> | 368.40 ± 16.06 <sup>abc</sup> |
| Rh-ZnSB 80 | 26.00 ± 2.03 <sup>ab</sup> | 69.00 ± 5.39 <sup>a</sup>  | 3.65 ± 0.29 <sup>de</sup>  | 265.40 ± 20.73 <sup>efg</sup> | 20.00 ± 1.56 <sup>bc</sup> | 70.00 ± 5.47 <sup>a</sup>  | 4.50 ± 0.35 <sup>bcd</sup>  | 350.00 ± 27.34 <sup>bcd</sup> |
| Rh-ZnSB 88 | 27.00 ± 2.11 <sup>ab</sup> | 65.00 ± 5.08 <sup>ab</sup> | 3.41 ± 0.27 <sup>e</sup>   | 240.70 ± 18.80 <sup>g</sup>   | 17.00 ± 1.33 <sup>d</sup>  | 58.00 ± 4.53 <sup>c</sup>  | 4.40 ± 0.34 <sup>cdef</sup> | 341.20 ± 26.65 <sup>cd</sup>  |
| Rh-ZnSB 90 | 28.00 ± 2.19 <sup>a</sup>  | 68.00 ± 5.31 <sup>a</sup>  | 3.43 ± 0.27 <sup>e</sup>   | 242.90 ± 18.97 <sup>fg</sup>  | 15.00 ± 1.17 <sup>e</sup>  | 57.00 ± 4.45 <sup>c</sup>  | 4.80 ± 0.37 <sup>abc</sup>  | 380.00 ± 29.68 <sup>ab</sup>  |
| Nd-ZnSB 1  | 20.00 ± 1.56 <sup>cd</sup> | 65.00 ± 5.08 <sup>ab</sup> | 4.25 ± 0.33 <sup>ab</sup>  | 325.00 ± 25.38 <sup>b</sup>   | 14.00 ± 1.09 <sup>e</sup>  | 55.00 ± 4.30 <sup>c</sup>  | 4.90 ± 0.38 <sup>ab</sup>   | 392.90 ± 30.69 <sup>a</sup>   |
| Nd-ZnSB 2  | 19.00 ± 1.48 <sup>de</sup> | 60.00 ± 4.69 <sup>b</sup>  | 4.16 ± 0.32 <sup>bc</sup>  | 315.80 ± 24.66 <sup>bc</sup>  | 20.00 ± 1.56 <sup>bc</sup> | 65.00 ± 5.08 <sup>ab</sup> | 4.30 ± 0.34 <sup>def</sup>  | 325.00 ± 25.38 <sup>de</sup>  |
| Nd-ZnSB 3  | 0.00 ± 0.00 <sup>f</sup>   | 0.00 ± 0.00 <sup>d</sup>   | 0.00 ± 0.00 <sup>f</sup>   | 0.00 ± 0.00 <sup>h</sup>      | 0.00 ± 0.00 <sup>f</sup>   | 0.00 ± 0.00 <sup>d</sup>   | 0.00 ± 0.00 <sup>g</sup>    | 0.00 ± 0.00 <sup>f</sup>      |
| CD         | 2.3                        | 6.4                        | 0.39                       | 29.15                         | 1.83                       | 6.31                       | 0.46                        | 35.63                         |
| CV( %)     | 6.88                       | 6.6                        | 6.42                       | 6.39                          | 6.52                       | 6.47                       | 6.53                        | 6.54                          |

FC: ferric chloride; FS: ferrous sulphate; CD: colony diameter; SD: siderophore diameter; FeSI: ferric siderophore index; FeSE: ferric siderophore efficiency; Rh-ZnSB: rhizosphere zinc-solubilising bacterial isolate; Nd-ZnSB: nodule-derived zinc-solubilising bacterial isolate; CD (statistical): critical difference ( $p \leq 0.05$ ); CV: coefficient of variation.

**Table 5.** Efficient zinc solubilising bacterial isolates on siderophore production at ferrous sulphate iron source

| Isolates  | FS – siderophore unit (%)  |                             |                            |                            |                            |                           |
|-----------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
|           | 1DAI                       | 3DAI                        | 5 DAI                      | 7DAI                       | 9DAI                       | 11 DAI                    |
| Rh-ZnSB 6 | 7.81 ± 0.29 <sup>i</sup>   | 23.00 ± 1.44 <sup>gh</sup>  | 28.00 ± 1.75 <sup>f</sup>  | 13.00 ± 0.81 <sup>h</sup>  | 35.00 ± 2.19 <sup>d</sup>  | 47.00 ± 2.94 <sup>c</sup> |
| Rh-ZnSB12 | 1.00 ± 0.05 <sup>j</sup>   | 21.10 ± 1.05 <sup>gh</sup>  | 29.00 ± 1.45 <sup>f</sup>  | 24.60 ± 1.23 <sup>g</sup>  | 30.00 ± 1.50 <sup>e</sup>  | 39.00 ± 1.95 <sup>d</sup> |
| Rh-ZnSB20 | 14.40 ± 0.88 <sup>h</sup>  | 12.00 ± 0.73 <sup>i</sup>   | 27.70 ± 1.68 <sup>f</sup>  | 28.80 ± 1.75 <sup>ef</sup> | 30.00 ± 1.82 <sup>e</sup>  | 37.70 ± 2.29 <sup>d</sup> |
| Rh-ZnSB38 | 48.80 ± 1.46 <sup>de</sup> | 19.00 ± 0.57 <sup>h</sup>   | 4.70 ± 0.14 <sup>h</sup>   | 32.50 ± 0.98 <sup>de</sup> | 40.00 ± 1.20 <sup>c</sup>  | 45.00 ± 1.35 <sup>c</sup> |
| Rh-ZnSB55 | 40.30 ± 1.76 <sup>f</sup>  | 39.00 ± 1.70 <sup>c</sup>   | 44.00 ± 1.92 <sup>c</sup>  | 34.00 ± 1.48 <sup>cd</sup> | 37.00 ± 1.61 <sup>cd</sup> | 57.00 ± 2.48 <sup>b</sup> |
| Rh-ZnSB60 | 25.30 ± 1.10 <sup>g</sup>  | 25.00 ± 1.09 <sup>efg</sup> | 28.90 ± 1.26 <sup>f</sup>  | 37.70 ± 1.64 <sup>c</sup>  | 39.00 ± 1.70 <sup>cd</sup> | 49.00 ± 2.14 <sup>c</sup> |
| Rh-ZnSB76 | 45.30 ± 1.97 <sup>e</sup>  | 28.00 ± 1.22 <sup>e</sup>   | 31.00 ± 1.35 <sup>ef</sup> | 34.00 ± 1.48 <sup>cd</sup> | 37.00 ± 1.61 <sup>cd</sup> | 48.00 ± 2.09 <sup>c</sup> |
| Rh-ZnSB80 | 55.90 ± 4.37 <sup>bc</sup> | 26.00 ± 2.03 <sup>ef</sup>  | 19.00 ± 1.48 <sup>g</sup>  | 26.60 ± 2.08 <sup>fg</sup> | 30.00 ± 2.34 <sup>e</sup>  | 32.00 ± 2.50 <sup>e</sup> |
| Rh-ZnSB88 | 51.90 ± 4.05 <sup>cd</sup> | 34.90 ± 2.73 <sup>d</sup>   | 34.00 ± 2.66 <sup>e</sup>  | 46.00 ± 3.59 <sup>a</sup>  | 48.00 ± 3.75 <sup>b</sup>  | 59.00 ± 4.61 <sup>b</sup> |
| Rh-ZnSB90 | 50.50 ± 3.94 <sup>d</sup>  | 48.90 ± 3.82 <sup>b</sup>   | 47.90 ± 3.74 <sup>b</sup>  | 48.80 ± 3.81 <sup>a</sup>  | 53.00 ± 4.14 <sup>a</sup>  | 75.00 ± 5.86 <sup>a</sup> |
| Nd-ZnSB 1 | 58.80 ± 4.59 <sup>b</sup>  | 48.90 ± 3.82 <sup>b</sup>   | 37.90 ± 2.96 <sup>d</sup>  | 42.00 ± 3.28 <sup>b</sup>  | 54.00 ± 4.22 <sup>a</sup>  | 57.00 ± 4.45 <sup>b</sup> |
| Nd-ZnSB 2 | 68.10 ± 5.32 <sup>a</sup>  | 66.60 ± 5.20 <sup>a</sup>   | 52.40 ± 4.09 <sup>a</sup>  | 48.30 ± 3.77 <sup>a</sup>  | 57.00 ± 4.45 <sup>a</sup>  | 59.00 ± 4.61 <sup>b</sup> |
| Nd-ZnSB 3 | 0.00 ± 0.00 <sup>j</sup>   | 0.00 ± 0.00 <sup>j</sup>    | 0.00 ± 0.00 <sup>i</sup>   | 0.00 ± 0.00 <sup>i</sup>   | 0.00 ± 0.00 <sup>f</sup>   | 0.00 ± 0.00 <sup>f</sup>  |
| CD        | 4.92                       | 4.09                        | 3.72                       | 3.9                        | 4.52                       | 5.46                      |
| CV( %)    | 8.14                       | 8.08                        | 7.49                       | 7.25                       | 7.14                       | 6.99                      |

FS: ferrous sulphate; DAI: days after inoculation; Rh-ZnSB: rhizosphere ZnSB isolate; Nd-ZnSB: nodule-derived ZnSB isolate; CD: critical difference ( $p \leq 0.05$ ); CV: coefficient of variation.

DAS, siderophore production by Rh-ZnSB isolates ranged from 55.80 ± 4.36 % to 78.00 ± 2.34 %. The highest value was recorded in Rh-ZnSB 38 (78.00 ± 2.34 %), followed by Rh-ZnSB 55 (73.90 ± 3.22 %) and Rh-ZnSB 60 (68.90 ± 3.00 %) Table 5 Fig.1e). Under ferrous sulphate (FS), siderophore production by Rh-ZnSB isolates ranged from 32.00 ± 2.50 % to 75.00 ± 5.86 %. The highest siderophore production was recorded in Rh-ZnSB 90 (75.00 ± 5.86 %), followed by Rh-ZnSB 88 (59.00 ± 4.61 %) and Rh-ZnSB 55 (57.00 ± 2.48 %) (Table 6; Fig.1e).

Among the Nd-ZnSB isolates, siderophore production at 11<sup>th</sup> DAS under FC ranged from 58.90 ± 4.60 % to 68.10 ± 5.32 %. The maximum production was observed in Nd-ZnSB 1 (68.10 ± 5.32 %), followed by Nd-ZnSB 2 (58.90 ± 4.60 %) (Table 5). Under FS ranged from 57.00 ± 4.45 % to 59.00 ± 4.61 %. The maximum production was recorded in Nd-ZnSB 2 (59.00 ± 4.61 %), followed closely by Nd-ZnSB 1 (57.00 ± 4.45 %), indicating strong siderophore-producing ability under ferrous sulphate (Table 6).

### Phytohormones and ammonia production of rhizospheric zinc-solubilising bacteria (Rh-ZnSB) and nodule associated zinc-solubilising bacteria isolates

The IAA production Among Rh-ZnSB isolates, IAA production ranged from 20.56 to 90.50 µg mL<sup>-1</sup> protein. The highest IAA production was recorded in Rh-ZnSB 90 (90.50 ± 7.07 µg mL<sup>-1</sup>), which was statistically at par with Rh-ZnSB 38 (89.56 ± 2.69 µg mL<sup>-1</sup>) and Rh-ZnSB 88 (88.30 ± 6.90 µg mL<sup>-1</sup>). In Nd-ZnSB isolates, IAA production ranged from 60.75 to 75.51 µg mL<sup>-1</sup>, with the maximum in Nd-ZnSB 1 (75.51 ± 5.90 µg mL<sup>-1</sup> protein) followed by Nd-ZnSB 3 (74.45 ± 5.81 µg mL<sup>-1</sup> protein) and Nd-ZnSB 2 (60.75 ± 4.74 µg mL<sup>-1</sup> protein) (Table 7).

For Rh-ZnSB isolates, GA<sub>3</sub> production varied from 45.19 to 180.74 µg mL<sup>-1</sup>. The highest GA<sub>3</sub> production was observed in Rh-ZnSB 60 (180.74 ± 7.88 µg mL<sup>-1</sup>), followed by Rh-ZnSB 38 (174.07 ± 5.22 µg mL<sup>-1</sup>) and Rh-ZnSB55 (172.59 ± 7.52 µg mL<sup>-1</sup>). In Nd-ZnSB isolates, GA<sub>3</sub> production ranged between 160.37 and 205.56 µg mL<sup>-1</sup>, highest GA<sub>3</sub> production were recorded in Nd-ZnSB 1 (205.56 ± 16.05 µg mL<sup>-1</sup>), followed by Nd-ZnSB 2 (164.44 ± 12.84 µg mL<sup>-1</sup>) and Nd-ZnSB 3 (160.37 ± 12.53 µg mL<sup>-1</sup>). All the rhizospheric and nodule isolates were observed positive in ammonia production (Table 7).

**Table 6.** Efficient zinc solubilising bacterial isolates on siderophore production at iron source of ferric chloride

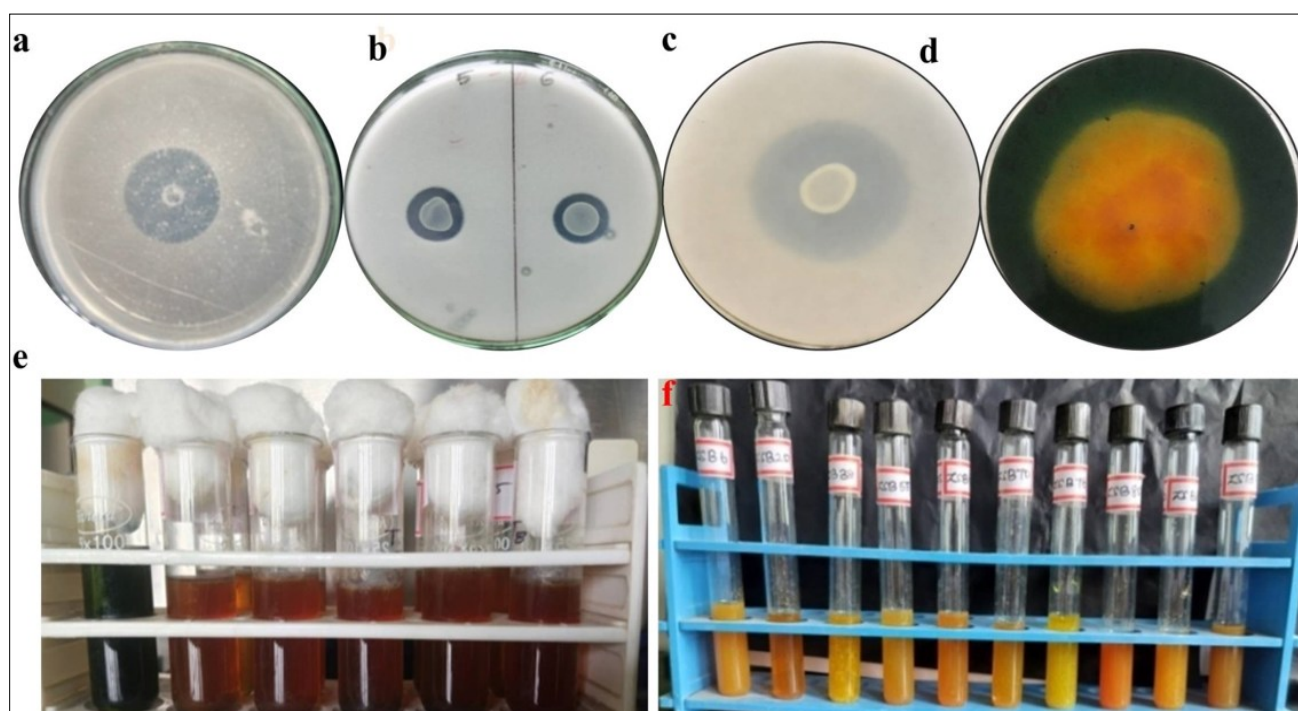
| Isolates  | FC – siderophore unit (%) |                           |                            |                            |                            |                            |
|-----------|---------------------------|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|           | 1DAI                      | 3DAI                      | 5 DAI                      | 7DAI                       | 9DAI                       | 11 DAI                     |
| Rh-ZnSB 6 | 38.80 ± 2.42 <sup>c</sup> | 28.90 ± 1.80 <sup>b</sup> | 24.80 ± 1.55 <sup>cd</sup> | 7.20 ± 0.45 <sup>e</sup>   | 50.00 ± 3.12 <sup>bc</sup> | 57.10 ± 3.57 <sup>d</sup>  |
| Rh-ZnSB12 | 27.50 ± 1.38 <sup>e</sup> | 18.60 ± 0.93 <sup>c</sup> | 13.60 ± 0.68 <sup>e</sup>  | 2.00 ± 0.10 <sup>hij</sup> | 46.00 ± 2.30 <sup>c</sup>  | 60.00 ± 3.00 <sup>d</sup>  |
| Rh-ZnSB20 | 34.80 ± 2.12 <sup>d</sup> | 8.40 ± 0.51 <sup>e</sup>  | 1.70 ± 0.10 <sup>gh</sup>  | 1.00 ± 0.06 <sup>ij</sup>  | 54.90 ± 3.34 <sup>ab</sup> | 56.80 ± 3.46 <sup>d</sup>  |
| Rh-ZnSB38 | 34.70 ± 1.04 <sup>d</sup> | 8.20 ± 0.25 <sup>e</sup>  | 2.50 ± 0.08 <sup>g</sup>   | 3.00 ± 0.09 <sup>ghi</sup> | 57.90 ± 1.74 <sup>a</sup>  | 78.00 ± 2.34 <sup>a</sup>  |
| Rh-ZnSB55 | 73.90 ± 3.22 <sup>a</sup> | 28.10 ± 1.22 <sup>b</sup> | 3.70 ± 0.16 <sup>g</sup>   | 4.00 ± 0.17 <sup>fgh</sup> | 35.90 ± 1.56 <sup>d</sup>  | 73.90 ± 3.22 <sup>ab</sup> |
| Rh-ZnSB60 | 62.80 ± 2.74 <sup>b</sup> | 27.80 ± 1.21 <sup>b</sup> | 6.90 ± 0.30 <sup>f</sup>   | 5.00 ± 0.22 <sup>efg</sup> | 57.00 ± 2.48 <sup>a</sup>  | 68.90 ± 3.00 <sup>bc</sup> |
| Rh-ZnSB76 | 62.60 ± 2.73 <sup>b</sup> | 37.60 ± 1.64 <sup>a</sup> | 37.20 ± 1.62 <sup>a</sup>  | 6.00 ± 0.26 <sup>ef</sup>  | 57.90 ± 2.52 <sup>a</sup>  | 66.90 ± 2.92 <sup>c</sup>  |
| Rh-ZnSB80 | 63.90 ± 4.99 <sup>b</sup> | 37.50 ± 2.93 <sup>a</sup> | 37.60 ± 2.94 <sup>a</sup>  | 18.20 ± 1.42 <sup>d</sup>  | 38.00 ± 2.97 <sup>d</sup>  | 55.80 ± 4.36 <sup>d</sup>  |
| Rh-ZnSB88 | 7.20 ± 0.56 <sup>f</sup>  | 12.00 ± 0.94 <sup>d</sup> | 25.70 ± 2.01 <sup>c</sup>  | 34.00 ± 2.66 <sup>c</sup>  | 49.00 ± 3.83 <sup>c</sup>  | 58.00 ± 4.53 <sup>d</sup>  |
| Rh-ZnSB90 | 1.10 ± 0.09 <sup>g</sup>  | 7.10 ± 0.55 <sup>e</sup>  | 23.00 ± 1.80 <sup>d</sup>  | 40.80 ± 3.19 <sup>b</sup>  | 55.90 ± 4.37 <sup>a</sup>  | 68.70 ± 5.37 <sup>bc</sup> |
| Nd-ZnSB 1 | 1.90 ± 0.15 <sup>g</sup>  | 7.90 ± 0.62 <sup>e</sup>  | 15.50 ± 1.21 <sup>e</sup>  | 43.90 ± 3.43 <sup>a</sup>  | 55.70 ± 4.35 <sup>a</sup>  | 68.10 ± 5.32 <sup>bc</sup> |
| Nd-ZnSB 2 | 6.90 ± 0.54 <sup>f</sup>  | 12.70 ± 0.99 <sup>d</sup> | 28.90 ± 2.26 <sup>b</sup>  | 39.00 ± 3.05 <sup>b</sup>  | 40.80 ± 3.19 <sup>d</sup>  | 58.90 ± 4.60 <sup>d</sup>  |
| Nd-ZnSB 3 | 0.00 ± 0.00 <sup>g</sup>  | 0.00 ± 0.00 <sup>f</sup>  | 0.00 ± 0.00 <sup>h</sup>   | 0.00 ± 0.00 <sup>i</sup>   | 0.00 ± 0.00 <sup>e</sup>   | 0.00 ± 0.00 <sup>e</sup>   |
| CD        | 3.73                      | 2.14                      | 2.47                       | 2.97                       | 5.01                       | 6.33                       |
| CV (%)    | 6.94                      | 7.07                      | 8.64                       | 11.26                      | 6.48                       | 6.36                       |

FC: ferric chloride; DAI: days after inoculation; Rh-ZnSB: rhizosphere ZnSBI isolate; Nd-ZnSB: nodule-derived ZnSBI isolate; CD: critical difference ( $p \leq 0.05$ ); CV: coefficient of variation.

**Table 7.** Evalutaion of efficient bacterial isolates on plant growth promoting traits

|            | IAA µg/mL                  | protein | GA Production µg/mL         | Ammonia production |
|------------|----------------------------|---------|-----------------------------|--------------------|
| Rh-ZnSB 6  | 70.56 ± 4.41 <sup>c</sup>  |         | 51.85 ± 3.24 <sup>fg</sup>  | +                  |
| Rh-ZnSB 12 | 78.57 ± 3.93 <sup>b</sup>  |         | 106.30 ± 5.31 <sup>d</sup>  | +                  |
| Rh-ZnSB 20 | 50.12 ± 3.05 <sup>e</sup>  |         | 96.30 ± 5.86 <sup>d</sup>   | +                  |
| Rh-ZnSB 38 | 89.56 ± 2.69 <sup>a</sup>  |         | 174.07 ± 5.22 <sup>bc</sup> | +                  |
| Rh-ZnSB 55 | 60.32 ± 2.63 <sup>d</sup>  |         | 172.59 ± 7.52 <sup>bc</sup> | +                  |
| Rh-ZnSB 60 | 59.52 ± 2.59 <sup>d</sup>  |         | 180.74 ± 7.88 <sup>b</sup>  | +                  |
| Rh-ZnSB 76 | 48.61 ± 2.12 <sup>e</sup>  |         | 75.93 ± 3.31 <sup>e</sup>   | +                  |
| Rh-ZnSB 80 | 20.56 ± 1.61 <sup>f</sup>  |         | 160.74 ± 12.55 <sup>c</sup> | +                  |
| Rh-ZnSB 88 | 88.30 ± 6.90 <sup>a</sup>  |         | 45.19 ± 3.53 <sup>g</sup>   | +                  |
| Rh-ZnSB 90 | 90.50 ± 7.07 <sup>a</sup>  |         | 65.93 ± 5.15 <sup>ef</sup>  | +                  |
| Nd-ZnSB 1  | 75.51 ± 5.90 <sup>bc</sup> |         | 205.56 ± 16.05 <sup>a</sup> | +                  |
| Nd-ZnSB 2  | 60.75 ± 4.74 <sup>d</sup>  |         | 164.44 ± 12.84 <sup>c</sup> | +                  |
| Nd-ZnSB 3  | 74.45 ± 5.81 <sup>bc</sup> |         | 160.37 ± 12.53 <sup>c</sup> | +                  |
| CD         | 7.52                       |         | 14.76                       |                    |
| CV (%)     | 6.71                       |         | 6.89                        |                    |

IAA: indole-3-acetic acid; GA: gibberellic acid; Rh-ZnSB: rhizosphere zinc-solubilising bacterial isolate; Nd-ZnSB: nodule-derived zinc-solubilising bacterial isolate; CD: critical difference ( $p \leq 0.05$ ); CV: coefficient of variation.

**Fig. 1.** Plant growth-promoting traits of the selected bacterial isolates: (a) zinc solubilisation; (b) phosphorus solubilisation; (c) potassium solubilisation; (d) siderophore production; (e) quantitative siderophore production; (f) ammonia production.

**Table 8.** Compatability test among rhizospheric zinc-solubilising bacteria (Rh-ZnSB) and nodule associated zinc-solubilising bacteria isolates

|           | Rh-ZnSB 6 | Rh-ZnSB 12 | Rh-ZnSB 20 | Rh-ZnSB 38 | Rh-ZnSB 55 | Rh-ZnSB 60 | Rh-ZnSB 76 | Rh-ZnSB 80 | Rh-ZnSB 88 | Rh-ZnSB 90 |
|-----------|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Nd ZnSB 1 |           |            |            |            |            |            |            |            |            |            |
| Nd ZnSB 2 |           |            |            |            |            |            |            |            |            |            |
| Nd ZnSB 3 |           |            |            |            |            |            |            |            |            |            |

Rh-ZnSB: rhizospheric zinc-solubilising bacteria; Nd-ZnSB: nodule-derived zinc-solubilising bacteria; ✓: compatibility between rhizospheric and nodule-derived zinc-solubilising bacterial isolates.

### Compatibility with *Rhizobium*

The compatibility of different microbial isolates was assessed using the cross-streaking method to determine their ability to coexist without antagonistic effects. The results demonstrated that both Rh-ZnSB and Nd- ZnSB isolates could grow in close proximity to each other without any observable inhibition. This indicates that all tested isolates exhibited high compatibility, suggesting their potential for co-inoculation as well as microbial consortia preparation and applications (Table 8).

### Consortium development

Based on the results of compatibility assessment and Zn solubilisation efficiency, 4 potential isolates- Rh-ZnSB 38, Rh-ZnSB 60, Rh-ZnSB 90 and Nd- ZnSB 1 were selected for consortium development. Compatibility testing revealed that the selected isolates exhibited no antagonistic interactions when co-cultured, indicating a high degree of mutual compatibility. This compatibility ensures their potential for synergistic interaction when applied together, supporting their use as a combined inoculant for enhancing lentil growth and iron solubilisation efficiency. Prior to consortium formulation, individual isolates were cultured in NB under aseptic conditions and incubated at  $28 \pm 2$  °C for 48 hr to achieve active growth. The bacterial cell density of each culture was adjusted to approximately  $1 \times 10^8$  CFU mL<sup>-1</sup> using a spectrophotometer (OD<sub>600</sub> = 0.5). Equal volumes of each isolate were then mixed in a 1 : 1 : 1 ratio to formulate a microbial consortium (Supplementary Fig. 1).

### Seed germination test for each isolate

Selected 4 cultures and 2 consortia (Nd-ZnSB 1, Rh-ZnSB 38, Rh-ZnSB 60, Rh- ZnSB 90, Rh- ZnSB Consortia, Nd- ZnSB 1 + Rh- ZnSB Consortia) were used to check the seed viability in early growth stage of plant. Among all treatments, the combined inoculation of Nd-ZnSB 1 with the Rh- ZnSB consortium (T7) exhibited the most significant enhancement in lentil seedling growth parameters compared to all other treatments. This treatment achieved 100 %

germination, the maximum shoot length ( $20.00 \pm 0.87$  cm) and root length ( $25.00 \pm 1.09$  cm) and the highest SVI of  $4500.00 \pm 196.15$ , corresponding to a 53.37 % increase over the uninoculated control (T1). The Rh-ZnSB consortium alone (T6) also demonstrated substantial growth promotion, recording an SVI of  $4200.00 \pm 183.07$  (43.15 % increase over control), followed by the mono nodule-associated isolate Nd-ZnSB 1 (T2), which achieved an SVI of  $3800.00 \pm 190.00$  (29.52 % increased). Among the individual-rhizospheric isolates, Rh-ZnSB 38 (T3) and Rh-ZnSB 82 (T4) significantly improved seedling vigor, with SVI values of 3700 and 3600, respectively, while Rh-ZnSB 90 (T4) (SVI  $3600.00 \pm 108.00$ ) at par with T4. The uninoculated control (T1) consistently recorded the lowest performance across all parameters (SVI  $2934.00 \pm 183.23$ ) (Fig. 2 a, Table 9).

### Pot experiment

#### Effect of zinc-solubilising bacteria isolates on plant biometric traits

Germination ranged from 90 % in the control (T<sub>1</sub>) to 100 % in all inoculated treatments (T<sub>2</sub>–T<sub>7</sub>), indicating that ZnSB inoculation positively influenced seed germination. Among all treatments, the highest germination (100 %) was recorded in T<sub>7</sub> (Nd ZnSB 1 + Rh ZnSB consortia), compared to 90 % in the control, showing an 11.11 % increase. T<sub>7</sub> also performed better than RDF, indicating improved seed establishment due to consortium inoculation under in vitro conditions.

Plant height was significantly influenced by microbial consortia under the microcosm experiment at both 45 days after sowing (DAS) and 90 DAS (Table 10). At 45 DAS, plant height ranged from 30.60 cm (T<sub>1</sub> – Control) to 38.80 cm (T<sub>7</sub>). T<sub>7</sub> showed the highest plant height, recording an increase of 26.80 % over the control and approximately 14.71 % over RDF. At 90 DAS, the maximum plant height (57.90 cm) was again observed in T<sub>7</sub>, which was significantly superior to all other treatments, followed by T<sub>6</sub> (54.30 cm) and T<sub>8</sub> (RDF, 52.40 cm). At this stage, T<sub>7</sub> showed an increase of 38.89 % over

**Table 9.** Evaluation of efficient zinc solubilising bacteria and consortia growth on lentil under *in vitro* condition

| Treatment | Germination   | SL                        | RL                         | SL+RL                     | SVI                           | IOC   |
|-----------|---------------|---------------------------|----------------------------|---------------------------|-------------------------------|-------|
| T1        | 90.00 ± 5.62  | 11.30 ± 0.71 <sup>d</sup> | 21.30 ± 1.33 <sup>c</sup>  | 32.60 ± 2.04 <sup>c</sup> | 2934.00 ± 183.23 <sup>c</sup> |       |
| T2        | 100.00 ± 5.00 | 15.00 ± 0.75 <sup>c</sup> | 23.00 ± 1.15 <sup>bc</sup> | 38.00 ± 1.90 <sup>b</sup> | 3800.00 ± 190.00 <sup>b</sup> | 29.52 |
| T3        | 100.00 ± 6.08 | 15.00 ± 0.91 <sup>c</sup> | 22.00 ± 1.34 <sup>c</sup>  | 37.00 ± 2.25 <sup>b</sup> | 3700.00 ± 225.06 <sup>b</sup> | 26.11 |
| T4        | 100.00 ± 4.36 | 14.00 ± 0.61 <sup>c</sup> | 22.00 ± 0.96 <sup>c</sup>  | 36.00 ± 1.57 <sup>b</sup> | 3600.00 ± 156.92 <sup>b</sup> | 22.70 |
| T5        | 100.00 ± 3.00 | 14.00 ± 0.42 <sup>c</sup> | 22.00 ± 0.66 <sup>c</sup>  | 36.00 ± 1.08 <sup>b</sup> | 3600.00 ± 108.00 <sup>b</sup> | 22.70 |
| T6        | 100.00 ± 4.36 | 18.00 ± 0.78 <sup>b</sup> | 24.00 ± 1.05 <sup>ab</sup> | 42.00 ± 1.83 <sup>a</sup> | 4200.00 ± 183.07 <sup>a</sup> | 43.15 |
| T7        | 100.00 ± 4.36 | 20.00 ± 0.87 <sup>a</sup> | 25.00 ± 1.09 <sup>a</sup>  | 45.00 ± 1.96 <sup>a</sup> | 4500.00 ± 196.15 <sup>a</sup> | 53.37 |
| SE(d)     | 0.60          | 0.90                      | 1.50                       | 147.55                    | 3.90                          |       |
| CD        | -             | 1.29                      | 1.93                       | 3.22                      | 316.47                        |       |
| CV (%)    | 4.84          | 4.82                      | 4.85                       | 4.83                      | 4.8                           |       |

SL: shoot length; RL: root length; SL + RL: total seedling length (shoot length + root length); SVI: seedling vigour index; IOC: index of colonisation; SE(d): standard error of difference; CD: critical difference ( $p \leq 0.05$ ); CV: coefficient of variation. Each value in a column represents the mean of three replications with  $\pm$  SD. Values sharing the same superscript alphabets differ significantly at  $p = 0.05$  according to Duncan's Multiple Range Test (DMRT). T1- Control; T2 - Nd ZnSB 1; T3- Rh- ZnSB 38; T4- Rh- ZnSB 60; T5- Rh- ZnSB 90; T6- Rh- ZnSB Consortia; T7- Nd- ZnSB 1 + Rh- ZnSB Consortia.

the control and 10.57 % over RDF. The lowest plant height at both growth stages was consistently recorded under the control treatment, indicating the positive effect of microbial consortia on plant growth.

The number of branches per plant was significantly influenced by microbial consortia at both 45 and 90 DAS. At 45 DAS, branch number varied from 3.20 in T<sub>1</sub> (control) to 5.60 in T<sub>7</sub> (Nd ZnSB 1 + Rh ZnSB consortia). The T<sub>7</sub> recorded the highest branching, followed by T<sub>6</sub> (5.10) and showed a marked improvement over the control (75.00 %) as well as over RDF. At 90 DAS, a similar trend was observed, where the highest number of branches was recorded in T<sub>7</sub> (8.90 plant<sup>-1</sup>), which was significantly superior to all other treatments. At this stage, T<sub>7</sub> exhibited an increase of 60.36 % over the control and 14.10 % over RDF. Treatments T<sub>6</sub> (8.20 plant<sup>-1</sup>) and T<sub>8</sub> (RDF, 7.80 plant<sup>-1</sup>) were statistically comparable, whereas the control consistently recorded the lowest branch number at both growth stages, confirming the beneficial effect of consortium inoculation on branching in lentil.

The number of nodules per plant showed a significant response to microbial consortia at both 45 and 90 DAS. At 45 DAS, nodulation ranged from 18.50 plant<sup>-1</sup> in T<sub>1</sub> (control) to 38.90 plant<sup>-1</sup> in T<sub>7</sub> (Nd ZnSB 1 + Rh ZnSB consortia). The highest nodulation was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (35.40 plant<sup>-1</sup>), indicating a clear improvement due to consortium inoculation over the control. At this stage, T<sub>7</sub> showed a marked increase over both control and RDF, confirming better nodulation under microbial treatment. At 90 DAS, a similar trend was observed, where T<sub>7</sub> produced the maximum nodules (45.60 plant<sup>-1</sup>), which was significantly higher than all treatments and statistically at par with T<sub>6</sub> (41.80 plant<sup>-1</sup>). The control consistently recorded the lowest nodulation at both growth stages, indicating the beneficial effect of microbial consortia on nodulation in lentil. Overall, T<sub>7</sub> remained the best-performing treatment at both stages in terms of nodule number development.

Nodule fresh and dry weights were significantly influenced by microbial consortia at both 45 and 90 DAS. At 45 DAS, nodule fresh weight ranged from 82.40 mg plant<sup>-1</sup> in T<sub>1</sub> (control) to 182.50 mg plant<sup>-1</sup> in T<sub>7</sub> (Nd ZnSB 1 + Rh ZnSB consortia). T<sub>7</sub> recorded the highest nodule fresh weight, followed by T<sub>6</sub> (168.20 mg plant<sup>-1</sup>), indicating a strong positive effect of consortium inoculation over the control. At 45 DAS, T<sub>7</sub> showed an increase of 121.36 % over the control and 8.46 % over RDF, respectively. At 90 DAS, a similar trend was observed, where the maximum nodule fresh weight (238.90 mg plant<sup>-1</sup>) was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (214.60 mg plant<sup>-1</sup>). At this stage, T<sub>7</sub> exhibited an increase of 60.64 % over the control and 11.27 % over RDF, respectively, confirming the consistent superiority of consortium treatments in enhancing nodule biomass in lentil.

Nodule dry weight also showed significant variation among treatments at both growth stages. At 45 DAS, the highest nodule dry weight (59.80 mg plant<sup>-1</sup>) was recorded in T<sub>7</sub> (Nd ZnSB 1 + Rh ZnSB consortia), which was statistically superior to all other treatments, followed by T<sub>6</sub> (53.60 mg plant<sup>-1</sup>). At this stage, T<sub>7</sub> showed an increase of 107.64 % over the control and 11.57 % over RDF, respectively. At 90 DAS, nodule dry weight further increased and reached a maximum of 80.40 mg plant<sup>-1</sup> in T<sub>7</sub>, followed by T<sub>6</sub> (71.20 mg plant<sup>-1</sup>), indicating improved nodulation and biomass accumulation under consortium inoculation. At this stage, T<sub>7</sub> exhibited an increase of 99.50 % over the control and 12.72 % over RDF, respectively (Fig.2b, Table 10).

**Table 10.** Effect of microbial consortia under microcosm experiment

| Treatments | Plant height (cm)          |                             | No of branches             |                            | No of nodules              |                             | Nodule fresh weight (mg/plant) |                              | Nodule dry weight (mg)      |                            |
|------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|--------------------------------|------------------------------|-----------------------------|----------------------------|
|            | 45DAS                      | 90DAS                       | 45DAS                      | 90DAS                      | 45DAS                      | 90DAS                       | 45DAS                          | 90DAS                        | 45DAS                       | 90DAS                      |
| T1         | 30.60 ± 1.91 <sup>c</sup>  | 39.80 ± 2.49 <sup>e</sup>   | 3.20 ± 0.20 <sup>f</sup>   | 5.40 ± 0.34 <sup>e</sup>   | 18.50 ± 1.16 <sup>e</sup>  | 22.60 ± 1.41 <sup>e</sup>   | 82.40 ± 5.15 <sup>e</sup>      | 104.30 ± 6.51 <sup>f</sup>   | 24.10 ± 1.51 <sup>f</sup>   | 31.50 ± 1.97 <sup>f</sup>  |
| T2         | 35.80 ± 2.24 <sup>ab</sup> | 46.20 ± 2.89 <sup>d</sup>   | 4.10 ± 0.26 <sup>e</sup>   | 6.80 ± 0.42 <sup>d</sup>   | 26.80 ± 1.67 <sup>d</sup>  | 31.40 ± 1.96 <sup>d</sup>   | 118.60 ± 7.41 <sup>d</sup>     | 156.80 ± 9.79 <sup>e</sup>   | 36.90 ± 2.30 <sup>e</sup>   | 48.70 ± 3.04 <sup>e</sup>  |
| T3         | 31.20 ± 1.95 <sup>c</sup>  | 48.90 ± 3.05 <sup>bcd</sup> | 4.40 ± 0.27 <sup>de</sup>  | 7.20 ± 0.45 <sup>cd</sup>  | 29.50 ± 1.84 <sup>cd</sup> | 34.80 ± 2.17 <sup>bcd</sup> | 132.40 ± 8.27 <sup>bcd</sup>   | 171.90 ± 10.74 <sup>de</sup> | 41.30 ± 2.58 <sup>cde</sup> | 54.60 ± 3.41 <sup>de</sup> |
| T4         | 32.60 ± 2.04 <sup>bc</sup> | 51.60 ± 3.22 <sup>bcd</sup> | 4.70 ± 0.29 <sup>bcd</sup> | 7.60 ± 0.47 <sup>bcd</sup> | 31.80 ± 1.99 <sup>c</sup>  | 37.90 ± 2.37 <sup>b</sup>   | 145.70 ± 9.10 <sup>b</sup>     | 189.40 ± 11.83 <sup>c</sup>  | 45.80 ± 2.86 <sup>c</sup>   | 61.80 ± 3.86 <sup>c</sup>  |
| T5         | 30.90 ± 1.93 <sup>c</sup>  | 47.80 ± 2.99 <sup>cd</sup>  | 4.30 ± 0.27 <sup>de</sup>  | 7.10 ± 0.44 <sup>cd</sup>  | 28.60 ± 1.79 <sup>cd</sup> | 33.60 ± 2.10 <sup>cd</sup>  | 126.90 ± 7.92 <sup>cd</sup>    | 165.20 ± 10.32 <sup>de</sup> | 39.40 ± 2.46 <sup>de</sup>  | 52.90 ± 3.30 <sup>de</sup> |
| T6         | 35.50 ± 2.22 <sup>ab</sup> | 54.30 ± 3.39 <sup>ab</sup>  | 5.10 ± 0.32 <sup>b</sup>   | 8.20 ± 0.51 <sup>ab</sup>  | 35.40 ± 2.21 <sup>b</sup>  | 41.80 ± 2.61 <sup>a</sup>   | 168.20 ± 10.50 <sup>a</sup>    | 214.60 ± 13.40 <sup>b</sup>  | 53.60 ± 3.35 <sup>b</sup>   | 71.20 ± 4.45 <sup>b</sup>  |
| T7         | 38.80 ± 2.42 <sup>a</sup>  | 57.90 ± 3.62 <sup>a</sup>   | 5.60 ± 0.35 <sup>a</sup>   | 8.90 ± 0.56 <sup>a</sup>   | 38.90 ± 2.43 <sup>a</sup>  | 45.60 ± 2.85 <sup>a</sup>   | 182.50 ± 11.40 <sup>a</sup>    | 238.90 ± 14.92 <sup>a</sup>  | 59.80 ± 3.73 <sup>a</sup>   | 80.40 ± 5.02 <sup>a</sup>  |
| T8         | 33.10 ± 2.07 <sup>bc</sup> | 52.40 ± 3.27 <sup>bc</sup>  | 4.80 ± 0.30 <sup>bc</sup>  | 7.80 ± 0.49 <sup>bc</sup>  | 30.20 ± 1.89 <sup>c</sup>  | 36.20 ± 2.26 <sup>bc</sup>  | 138.60 ± 8.66 <sup>bc</sup>    | 181.30 ± 11.32 <sup>cd</sup> | 43.70 ± 2.73 <sup>cd</sup>  | 58.60 ± 3.66 <sup>cd</sup> |
| CD         | 3.64                       | 5.42                        | 0.49                       | 0.8                        | 3.3                        | 3.9                         | 15.12                          | 19.64                        | 4.78                        | 6.39                       |
| CV (%)     | 6.27                       | 6.28                        | 6.31                       | 6.3                        | 6.36                       | 6.35                        | 6.38                           | 6.38                         | 6.41                        | 6.42                       |

Each value in a column represents the mean of three replications with ± SD. Values sharing the same superscript alphabets differ significantly at  $p = 0.05$  according to Duncan's Multiple Range Test (DMRT). DAS: days after sowing; CD: critical difference ( $p \leq 0.05$ ); CV: coefficient of variation; SD: standard deviation; DMRT: Duncan's multiple range test. Each value represents the mean of three replications ± standard deviation. Within each column, means followed by different lowercase letters differ significantly at  $p \leq 0.05$  according to DMRT.



**Fig. 2.** Microcosm study: (a) Roll towel method; (b) Pot experiment.

#### Effect of zinc-solubilising bacteria on yield parameters

The number of pods per plant was significantly influenced by ZnSB consortia under the microcosm experiment. The range was recorded from 50.00 to 75.00 plant<sup>-1</sup>. Among the treatment the highest number of pods was recorded in T<sub>7</sub> (75.00 plant<sup>-1</sup>), which was statistically superior to all other treatments, followed by T<sub>8</sub> (70.00 plant<sup>-1</sup>) and T<sub>6</sub> (68.00 plant<sup>-1</sup>). The lowest number of pods was observed in the control (T<sub>1</sub>; 50.00 plant<sup>-1</sup>). When Compared to the control, T<sub>7</sub> recorded a 50.00 % increase in pod number, while it showed a 7.14 % increase over RDF (T<sub>8</sub>) (Table 11).

The number of seeds per plant varied from 69.00 to 130.00 plant<sup>-1</sup>. The maximum number of seeds was recorded in T<sub>7</sub> (130.00 plant<sup>-1</sup>), which remained statistically at par with T<sub>8</sub> (128.00 plant<sup>-1</sup>) and T<sub>2</sub> (120.00 plant<sup>-1</sup>). The control treatment recorded the lowest number of seeds (69.00 plant<sup>-1</sup>). The number of seeds in T<sub>7</sub> was 88.41 % higher than the control and 1.56 % higher than RDF (Table 11).

A significant variation was observed in 100-seed weight was observed among treatments, ranging from 2.40 to 5.30 g. The highest 100-seed weight was recorded in T<sub>7</sub> (5.30 g), followed by T<sub>2</sub> and T<sub>8</sub> (4.70 g). The lowest value was observed in the control (2.40 g). Compared to the control, T<sub>7</sub> exhibited a 120.83 % increase in 100-seed weight and a 12.77 % increase over RDF (Table 11).

Grain yield per pot ranged from 15.00 to 30.00 g pot<sup>-1</sup>. The highest grain yield was recorded in T<sub>7</sub> (30.00 g pot<sup>-1</sup>), which was statistically superior to all other treatments, followed by T<sub>8</sub> (24.00 g pot<sup>-1</sup>) and T<sub>2</sub> (22.00 g pot<sup>-1</sup>). The lowest grain yield was observed in the control (15.00 g pot<sup>-1</sup>). Compared to the control, T<sub>7</sub> recorded a 100.00 % increase in grain yield, while it showed a 25.00 % increase over RDF (Table 11).

#### Effect of zinc-solubilising bacteria in mono and consortium in grain zinc and iron content

Lentil grain zinc concentration varied significantly among treatments. The highest grain Zn content (46.80 mg kg<sup>-1</sup>) was recorded in T<sub>7</sub>, which was statistically superior to all other treatments. This was followed by T<sub>6</sub> (41.50 mg kg<sup>-1</sup>), while the lowest grain Zn content (21.40 mg kg<sup>-1</sup>) was observed in the control (Fig.3). Grain iron content was significantly enhanced by ZnSB consortia application. The maximum grain Fe content (102.30 mg kg<sup>-1</sup>) was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (94.80 mg kg<sup>-1</sup>). The lowest grain Fe concentration (62.50 mg kg<sup>-1</sup>) was observed in the control treatment (Fig. 3).

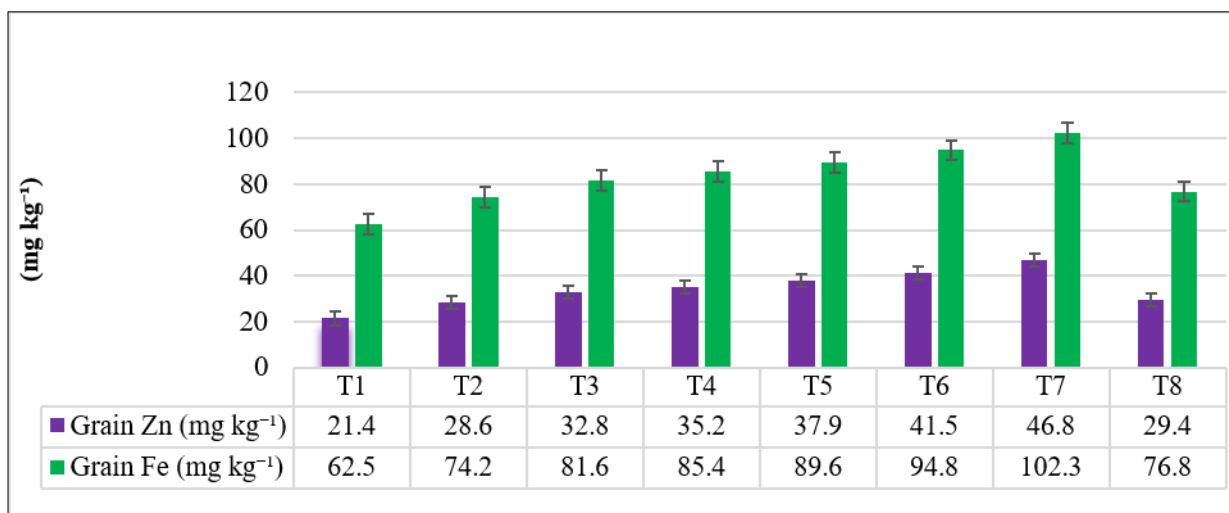
#### Effect of zinc-solubilising bacteria in mono and consortium in soil nutrient content

Available soil nitrogen was significantly influenced by ZnSB consortia. The highest available nitrogen (1.18 g kg<sup>-1</sup>) was recorded in T<sub>7</sub>, which was statistically superior to all other treatments. This was followed by T<sub>6</sub> (1.10 g kg<sup>-1</sup>) and T<sub>8</sub> (1.08 g kg<sup>-1</sup>), while the lowest nitrogen content (0.78 g kg<sup>-1</sup>) was observed in the control (T<sub>1</sub>) (Fig. 4). Soil available phosphorus increased significantly with ZnSB consortia application. The maximum phosphorus content (0.64 g kg<sup>-1</sup>) was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (0.59 g kg<sup>-1</sup>). The minimum available phosphorus (0.32 g kg<sup>-1</sup>) was observed under the control treatment (Fig.4). Available potassium content varied significantly among treatments. The highest potassium level (1.56 g kg<sup>-1</sup>) was recorded in T<sub>7</sub>, which was statistically superior to other treatments, followed by T<sub>6</sub> (1.49 g kg<sup>-1</sup>) and T<sub>8</sub> (1.52 g kg<sup>-1</sup>). The control treatment recorded the lowest available potassium (1.15 g kg<sup>-1</sup>) (Fig. 4). Soil organic carbon content was significantly enhanced by ZnSB consortia. The maximum organic carbon (0.66 %) was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (0.61 %), while the lowest organic carbon content

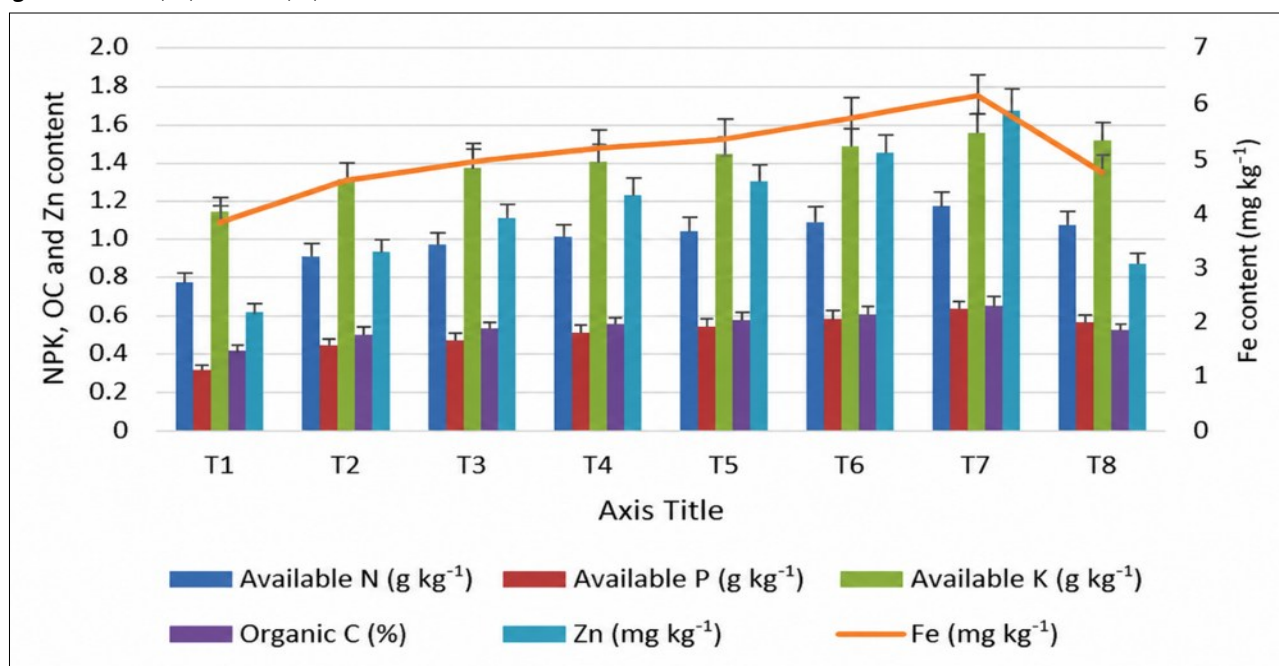
**Table 11.** Effect of zinc-solubilising bacteria consortia of lentil yield parameters under microcosm experiment

| Treatment | No. of pods per plant      | No. of seeds per plant     | 100 seed weight (g plant <sup>-1</sup> ) | Grain yield per pot (g pot <sup>-1</sup> ) |
|-----------|----------------------------|----------------------------|------------------------------------------|--------------------------------------------|
| T1        | 50.00 ± 3.12 <sup>c</sup>  | 69.00 ± 4.31 <sup>c</sup>  | 2.40 ± 0.15 <sup>f</sup>                 | 15.00 ± 0.94 <sup>f</sup>                  |
| T2        | 65.00 ± 4.06 <sup>b</sup>  | 120.00 ± 7.49 <sup>a</sup> | 4.70 ± 0.29 <sup>b</sup>                 | 22.00 ± 1.37 <sup>bc</sup>                 |
| T3        | 53.00 ± 3.31 <sup>c</sup>  | 89.00 ± 5.56 <sup>b</sup>  | 4.00 ± 0.25 <sup>c</sup>                 | 19.00 ± 1.19 <sup>de</sup>                 |
| T4        | 55.00 ± 3.43 <sup>c</sup>  | 86.00 ± 5.37 <sup>b</sup>  | 3.80 ± 0.24 <sup>cd</sup>                | 18.00 ± 1.12 <sup>e</sup>                  |
| T5        | 64.00 ± 4.00 <sup>b</sup>  | 92.00 ± 5.75 <sup>b</sup>  | 3.50 ± 0.22 <sup>de</sup>                | 20.00 ± 1.25 <sup>cde</sup>                |
| T6        | 68.00 ± 4.25 <sup>b</sup>  | 96.00 ± 6.00 <sup>b</sup>  | 3.30 ± 0.21 <sup>e</sup>                 | 21.00 ± 1.31 <sup>cd</sup>                 |
| T7        | 75.00 ± 4.68 <sup>a</sup>  | 130.00 ± 8.12 <sup>a</sup> | 5.30 ± 0.33 <sup>a</sup>                 | 30.00 ± 1.87 <sup>a</sup>                  |
| T8        | 70.00 ± 4.37 <sup>ab</sup> | 128.00 ± 7.99 <sup>a</sup> | 4.70 ± 0.29 <sup>b</sup>                 | 24.00 ± 1.50 <sup>b</sup>                  |
| SE(d)     | 6.82                       | 11.17                      | 0.44                                     | 2.33                                       |
| CD        | 6.30                       | 6.37                       | 6.39                                     | 6.37                                       |

SE(d): standard error of difference; CD: critical difference ( $p \leq 0.05$ ). Data are presented as mean ± standard deviation (SD).



**Fig. 3.** Grain zinc (Zn) and iron (Fe) contents under different treatments.



**Fig. 4.** Soil nutrient status.

(0.42 %) was observed in the control treatment. DTPA-extractable soil zinc increased significantly due to ZnSB consortia application (Fig. 4). The highest soil Zn content (1.68 mg kg<sup>-1</sup>) was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (1.46 mg kg<sup>-1</sup>). The lowest soil Zn (0.62 mg kg<sup>-1</sup>) was observed in the control (Fig.4). Soil iron content was significantly affected by ZnSB consortia. The maximum DTPA-extractable Fe (6.12 mg kg<sup>-1</sup>) was recorded in T<sub>7</sub>, followed by T<sub>6</sub> (5.72 mg kg<sup>-1</sup>), whereas the control treatment recorded the lowest soil Fe content (3.85 mg kg<sup>-1</sup>) (Fig. 4).

## Discussion

As a result of the isolation and screening of bacteria from the rhizosphere and nodules of lentil, the predominance of Gram-negative bacteria among the zinc-solubilising isolates observed in the present study corroborates earlier reports indicating that members of *Pseudomonas*, *Burkholderia*, *Enterobacter* and related genera dominate legume rhizospheres and possess strong mineral-solubilising abilities (51, 52). The observed morphological diversity and pigmentation among the isolates further suggest enhanced ecological adaptability, stress tolerance and secondary metabolite production. Such traits have been previously associated with

improved siderophore secretion and organic acid production, which facilitate micronutrient mobilisation under nutrient-limited soil conditions (53, 54).

Zinc solubilisation patterns on ZnO and ZnCO<sub>3</sub> media observed in the present study are consistent with earlier findings indicating that ZnO is more readily solubilised than ZnCO<sub>3</sub>, owing to differences in mineral stability and microbial acidification capacity (55, 56). The observed increase in soluble zinc up to 7–9 days after inoculation, followed by a subsequent decline, may be attributed to initial organic acid production, stabilisation of medium pH and possible re-precipitation or immobilisation of zinc complexes (56, 57), highlighting the time-dependent dynamics of microbial zinc mobilisation.

The qualitative zinc solubilisation assay revealed that a subset of isolates exhibited strong Zn-SE across both ZnO and ZnCO<sub>3</sub> media, demonstrating their functional ability to mobilise zinc from multiple inorganic sources. In the present study, Zn-SE (Zn-SE) ranged from 25.00 to 416.70 % on ZnO and 20.00 to 271.43 % on ZnCO<sub>3</sub>. Among the tested isolates, Rh-ZnSB 38 (416.70 ± 32.54 %) and Nd-ZnSB 1 (357.10 ± 27.89 %) exhibited the highest Zn-SE, indicating superior zinc-mobilising potential. These findings are in

close agreement with earlier reports documenting source-dependent variability in zinc solubilisation efficiency. Pronounced variation in Zn-SE values has been reported, ranging from 273.68–411.76 % for zinc phosphate, 160.00–250.00 % for ZnO and 177.78–244.44 % for ZnCO<sub>3</sub>, which closely aligns with the trends observed in the present investigation (58). Similarly, high solubilisation efficiencies across different zinc sources, including Zn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (243.5–305.5 %), ZnO (292.3–299.1 %) and ZnCO<sub>3</sub> (240.2–310.8 %), have also been documented (59). Earlier studies reported Zn-SE values of 214.28 % on ZnO (60), while others recorded Zn-SE ranges from 123–233 % for ZnO, 106–200 % for zinc phosphate and 114–159 % for zinc carbonate, further confirming source-specific zinc solubilisation behaviour (61). In addition, wide variability in Zn-SE among rhizobacterial isolates under laboratory conditions has been reported, with Zn-SE ranging from 120.3–226.8 % for ZnO, 115.7–206.0 % for ZnCO<sub>3</sub>, 110.5–223.0 % for ZnPO<sub>4</sub> and 107.8–201.7 % for ZnS (62). Collectively, these studies emphasize the influence of zinc mineral form on microbial solubilisation efficiency and strongly support the potential application of efficient ZnSB for enhancing zinc bioavailability in agricultural soils.

Quantitative assessment revealed that zinc solubilisation by rhizospheric and nodule bacterial isolates varied markedly with the zinc substrate. In ZnO-amended medium, halo diameters ranged from 2.51 ± 0.20 to 3.93 ± 0.12 mm, with Rh-ZnSB 38 (3.93 ± 0.12 mm), Rh-ZnSB 60 (3.79 ± 0.17 mm) and Nd-ZnSB 1 (3.77 ± 0.29 mm) exhibiting the highest solubilisation. Under ZnCO<sub>3</sub> conditions, solubilisation ranged from 1.92 ± 0.15 to 3.51 ± 0.11 mm in rhizospheric isolates and 1.26 ± 0.18 to 3.28 ± 0.26 mm in nodule isolates, with Rh-ZnSB 38 (3.51 ± 0.11 mm) and Nd-ZnSB 1 (3.28 ± 0.26 mm) showing maximum activity. Overall, ZnO proved to be a more favorable substrate than ZnCO<sub>3</sub>, likely due to enhanced proton extrusion and organic acid-mediated acidification mechanisms, which promote zinc mobilisation (55). The values obtained in the present study are in close agreement with earlier reports documenting substrate-dependent variability in zinc solubilisation. Previous studies have reported substantial variation in solubilised zinc concentrations and halo diameters across ZnO, ZnCO<sub>3</sub> and Zn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> media, with halo diameters typically ranging from 2.0–4.0 mm and high solubilisation efficiencies for efficient *Bacillus*, *Pseudomonas* and *Rhizobium* strains isolated from legume rhizospheres (59, 25).

The presence of multiple plant growth-promoting traits, including siderophore production, phosphate, potassium solubilisation, phytohormone production (IAA, GA) and ammonia release among the selected isolates, highlights their multifunctional nature and agrees with previous studies emphasising that efficient PGPR typically express multiple beneficial traits simultaneously (63, 57, 64). The production of phytohormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA<sub>3</sub>) by Zn-solubilising bacterial isolates highlights their strong plant growth-promoting potential. Among the isolates, Rh-ZnSB 90 (90.50 ± 7.07 µg mL<sup>-1</sup>) and Nd-ZnSB (75.51 ± 5.90 µg mL<sup>-1</sup> protein) exhibited the highest IAA production, while GA<sub>3</sub> production ranged from 51.85 ± 3.24 to 205.56 ± 16.05 µg mL<sup>-1</sup>, indicating robust auxin and gibberellin biosynthetic capacity. The higher IAA production recorded in Rh-ZnSB 90 and Nd-ZnSB, along with the elevated GA<sub>3</sub> production observed among the selected isolates, may be attributed to their efficient phytohormone biosynthetic activity, which promotes root elongation, cell division and nutrient uptake. These values fall within or exceed the ranges reported for efficient PGPR isolates, where IAA production typically

varies between 15–80 µg mL<sup>-1</sup> and GA<sub>3</sub> between 100–300 µg mL<sup>-1</sup> in legume-associated rhizobacteria (65, 66). Other studies have reported IAA production ranging from 45.66 to 111.94 µg mL<sup>-1</sup> (67). Such hormone levels are known to promote root elongation, lateral root initiation and nodulation in legumes, thereby enhancing nutrient uptake and overall plant vigor. The increased root length observed in inoculated seedlings (up to 46.8 cm) further corroborates the hormonal influence on root development, consistent with previous findings in IAA-producing PGPR associated with lentil and mungbean (68–70).

Phosphate solubilisation patterns observed in the present study revealed considerable variation between plate-based and liquid assays, which agrees with earlier findings that halo zone formation on solid media does not always accurately reflect phosphate solubilisation efficiency under liquid conditions. This discrepancy is commonly attributed to differences in organic acid diffusion, pH dynamics, microbial growth behavior and assay sensitivity (71, 72). In the present study, superior phosphate solubilisation indices under plate assay conditions were exhibited by Rh-ZnSB (905.18 ± 40.48 %) and Nd-ZnSB-1 (503.51 ± 39.33 %). Quantitative estimation under liquid culture conditions further revealed that Rh-ZnSB-60 (2.02 ± 0.09 mg mL<sup>-1</sup>) and Nd-ZnSB-1 (2.30 ± 0.18 mg mL<sup>-1</sup>) recorded the highest concentrations of soluble phosphorus. The superior phosphate-solubilising ability of Rh-ZnSB 60 and Nd-ZnSB 1 may be due to their enhanced production of organic acids, which convert insoluble phosphorus into plant-available forms and improve nutrient acquisition. These results are consistent with previous reports documenting phosphate solubilisation efficiencies ranging from 280 to 520 % and soluble phosphorus levels of 1.0–2.6 mg mL<sup>-1</sup> in efficient phosphate-solubilizing PGPR, including *Bacillus*, *Pseudomonas* and *Rhizobium* spp. associated with pulse crops (51, 27). Comparable solubilisation capacities have also been reported by previous researchers who observed soluble phosphorus production by *Bacillus mycoides* (1.09 ± 0.00 mg mL<sup>-1</sup>) and *Priestia aryabhatai* (1.10 ± 0.05 mg mL<sup>-1</sup>) (33), further corroborating the efficiency of the isolates evaluated in the present study. The enhanced phosphate solubilisation observed under liquid conditions aligns with earlier studies demonstrating that certain *Rhizobium* strains possess efficient gluconic acid and 2-ketogluconic acid production pathways, which facilitate the effective solubilisation of insoluble phosphate compounds (73, 74).

Potassium solubilisation by Rh-ZnSB (210.00 ± 9.15 to 333.30 ± 14.53) and Nd-ZnSB isolates (155.60 ± 12.15 to 266.70 ± 20.83) observed in the present study supports earlier reports that silicate- and feldspar-solubilising bacteria mobilise potassium through acidolysis, chelation and complexation mechanisms (75). Significantly higher potassium release was recorded in Rh-ZnSB-60 (333.30 ± 14.53) and Nd-ZnSB-1 (266.70 ± 20.83). Quantitative estimation on the 7<sup>th</sup> day after inoculation revealed soluble potassium concentrations of 13.14 ± 0.57 to 19.12 ± 1.49 mg mL<sup>-1</sup> in rhizospheric isolates and 14.82 ± 1.16 to 19.12 ± 1.49 mg mL<sup>-1</sup> in nodule isolates, indicating strong K-releasing ability. The highest potassium-solubilising activity recorded in Rh-ZnSB 60 and Nd-ZnSB 1 may be attributed to their ability to release organic acids and chelating compounds that mobilise potassium from insoluble mineral sources. These results agree with previous findings showing enhanced potassium availability and crop performance due to K-solubilising bacteria; K-solubilisation zones ranging from 15–32 mm

have been previously reported (52), while comparable potassium-solubilising activity was also observed in *Priestia aryabhatai* (33).

Enhanced siderophore production by both rhizospheric and nodule ZnSBI isolates under  $\text{FeCl}_3$  and  $\text{FeSO}_4$  conditions indicates their strong potential for improving iron availability in the rhizosphere. In the present study, qualitative siderophore production (Fe-SE) in rhizospheric isolates ranged from  $240.70 \pm 18.80$  to  $361.10 \pm 10.83$  % ( $\text{FeCl}_3$ ) and  $304.50 \pm 13.27$  to  $400.00 \pm 12.00$  % ( $\text{FeSO}_4$ ), while nodule isolates showed values of  $315.80 \pm 24.66$  to  $325.00 \pm 25.38$  % ( $\text{FeCl}_3$ ) and  $325.00 \pm 25.38$  to  $392.90 \pm 30.69$  % ( $\text{FeSO}_4$ ).

Quantitative siderophore production in rhizospheric isolates ranged from  $55.80 \pm 4.36$  to  $78.00 \pm 2.34$  % ( $\text{FeCl}_3$ ) and  $32.00 \pm 2.50$  to  $75.00 \pm 5.86$  % ( $\text{FeSO}_4$ ), whereas nodule isolates recorded  $58.90 \pm 4.60$  to  $68.10 \pm 5.32$  % ( $\text{FeCl}_3$ ) and  $57.00 \pm 4.45$  to  $59.00 \pm 4.61$  % ( $\text{FeSO}_4$ ). The greater siderophore production exhibited by Rh-ZnSB 90 and Nd-ZnSB 1 indicates their strong ability to chelate Fe, thereby enhancing iron availability in the rhizosphere and supporting plant growth. These results are consistent with earlier findings reporting high qualitative and quantitative siderophore production, ranging from  $202.6 \pm 2.334$  to  $265.0 \pm 1.362$  % qualitatively and  $12.34 \pm 0.105$  to  $23.55 \pm 0.256$  % quantitatively (76), as well as siderophore units of 89.97 % and 79.90 % reported in biofortification studies (77), supporting the role of siderophore-producing PGPR in enhancing iron and zinc availability.

The absence of antagonistic interactions between ZnSBI isolates and nodule bacterial isolates confirms their compatibility and suitability for consortium development. This observation agrees with earlier studies demonstrating that co-inoculation of mineral-solubilising bacteria with nodule isolates enhances nodulation, biological nitrogen fixation, nutrient uptake and overall plant growth in legumes (78, 67). The superior performance of microbial consortia in improving seed germination and seedling vigor observed in the present study further validates previous reports highlighting synergistic interactions between *Rhizobium* and zinc-solubilising PGPR in lentil, chickpea and mungbean (79, 80).

Microbial consortia significantly influenced plant growth and nodulation under the microcosm experiment in the present study. Plant height ranged from 30.60–38.80 cm at 45 DAS and 42.10–57.90 cm at 90 DAS, while the number of branches varied from 3.20–5.60 plant<sup>-1</sup> at 45 DAS and 5.10–8.90 plant<sup>-1</sup> at 90 DAS. The number of nodules ranged from 18.50–38.90 plant<sup>-1</sup> at 45 DAS and 24.30–45.60 plant<sup>-1</sup> at 90 DAS. Likewise, nodule fresh weight ranged from 82.40–182.50 mg plant<sup>-1</sup> at 45 DAS and 126.80–238.90 mg plant<sup>-1</sup> at 90 DAS, while nodule dry weight varied from 28.60–59.80 mg plant<sup>-1</sup> and 42.30–80.40 mg plant<sup>-1</sup> at 45 and 90 DAS, respectively. The superior performance of T<sub>7</sub> may be attributed to the synergistic interaction among the microbial consortium members, which enhanced nutrient availability through zinc, phosphorus and potassium solubilisation while simultaneously producing plant growth-promoting substances. Improved nutrient acquisition and phytohormone production likely stimulated root growth, leading to greater nodulation, nodule biomass accumulation and overall plant development. These results are in close agreement with earlier studies reporting significant enhancement of growth and nodulation attributes due to combined application of biofertilisers and nutrients, where plant height ranged from 51.81 to 54.94 cm, number of nodules from 31.68 to 36.28 plant<sup>-1</sup> and plant dry weight

from 18.45 to 23.71 g under integrated treatments (81). Similarly, bacterial inoculation under field conditions was reported to improve root length (8.10–9.90 cm), plant height (26.89–29.91 cm), number of branches (2.82–3.53 plant<sup>-1</sup>) and nodulation (7.09–9.30 nodules plant<sup>-1</sup>) in lentil (82). In addition, significant enhancement of nodulation in lentil cultivars due to rhizobial strains has been reported, with nodule number reaching up to 23.233 plant<sup>-1</sup>, nodule size up to 0.1022 cm and nodule weight up to 20.034 mg plant<sup>-1</sup> (83).

Under the microcosm experiment, ZnSB consortia significantly enhanced yield and yield-attributing characters of lentil. The number of pods per plant ranged from 50.00 to 75.00, number of seeds per plant from 69.00 to 130.00 and 100-seed weight from 2.40 to 5.30 g across treatments. Grain yield per pot also varied markedly, ranging from 15.00 to 30.00 g, with ZnSB consortia treatments consistently recording the highest values, indicating a strong positive influence on productivity.

The enhanced yield performance of T<sub>7</sub> may also be attributed to the synergistic interaction between Nd-ZnSB 1 and the Rh-ZnSB consortia, which likely improved rhizosphere colonisation and nutrient acquisition efficiency. The increased nodulation and nodule biomass observed under T<sub>7</sub> may have enhanced biological nitrogen fixation, resulting in a greater supply of nitrogen during critical growth stages. In addition, improved root development could have increased the absorption of water and essential nutrients, supporting higher photosynthetic activity and dry matter accumulation. These combined effects may have contributed to greater flower retention, pod formation, seed development and ultimately higher grain yield in lentil. These findings are in close agreement with earlier reports demonstrating significant variation in lentil yield attributes under consortium-based inoculation (28). In that study, the number of pods per plant increased from 25–30 in the control to 80–85 under consortium treatments, while seed yield per pot improved from 20–22 g in the control to 30–32 g under consortium application. The number of seeds per pod increased from 1.2–1.3 in the control to 1.9–2.0 under consortium treatments and 100-seed weight ranged from 2.4–2.5 g in the control to 3.4–3.5 g under microbial consortia. Furthermore, pod number varied from 20.0 pods plant<sup>-1</sup> in the control to 95.33 pods plant<sup>-1</sup> under consortium treatment. Overall, *Rhizobium*-based microbial consortia, particularly combinations involving *Rhizobium*, *Bacillus*, *Pseudomonas* and *Azotobacter*, consistently outperformed individual *Rhizobium* inoculation in enhancing lentil yield (28).

In the present study, ZnSB consortia application markedly enhanced soil fertility, with available nitrogen ranging from 0.78 to 1.18 g kg<sup>-1</sup>, phosphorus from 0.32 to 0.64 g kg<sup>-1</sup> and potassium from 1.15 to 1.56 g kg<sup>-1</sup> across treatments. The highest values of N, P and K were consistently recorded under treatment T<sub>7</sub>, indicating superior nutrient mobilisation under microbial consortia application. These findings are in close agreement with earlier reports showing a positive improvement in soil nutrient status following lentil cultivation, where total nitrogen increased from 0.40 to 0.50 g kg<sup>-1</sup>, available phosphorus from 10.67 to 11.77 mg kg<sup>-1</sup> and available potassium from 277 to 285 mg kg<sup>-1</sup>, highlighting the beneficial effect of PGPR on soil NPK dynamics (84). Similar trends were also reported under integrated nutrient management strategies involving PGPR, chemical fertilisers and organic amendments, resulting in significant increases in soil nitrogen, phosphorus and potassium content over the control (40). The consistency in nutrient enhancement trends across these studies

underscores the role of beneficial microorganisms in improving nutrient solubilisation, mineralisation and retention in legume-based systems. Furthermore, the positive response observed in the present study aligns with reports demonstrating improved crop performance and productivity under combined application of rhizobial and phosphate-solubilising bioinoculants, resulting in a 13.82–15.52 % increase in yield over the control (85). The comparable improvements in soil nutrient availability and crop performance highlight the synergistic effects of microbial inoculants in enhancing nutrient use efficiency, soil organic carbon accumulation and sustainable crop productivity.

In this present study, Soil organic carbon content varied significantly among treatments in the present study, ranging from 0.42 % in the control ( $T_1$ ) to 0.66 % in treatment  $T_7$ , with a progressive increase observed under ZnSB consortia treatments, particularly  $T_6$  (0.61 %) and  $T_7$  (0.66 %), indicating improved soil carbon status due to enhanced microbial activity. These findings are in close agreement with the previous reports which observed that inoculation with *Rhizobium* improved soil quality by increasing organic carbon (organic matter) from 1.22–1.23 % to 1.47 % in inoculated soils (86).

Diethylenetriaminepentaacetic acid (DTPA)-extractable soil zinc and iron contents were significantly enhanced by the application of ZnSB consortia in the present study. Soil zinc availability ranged from 0.62 to 1.68 mg kg<sup>-1</sup>, with the highest Zn content recorded in treatment  $T_7$ , followed by  $T_6$  (1.46 mg kg<sup>-1</sup>), while the control treatment exhibited the lowest zinc availability. Similarly, DTPA-extractable soil iron varied from 3.85 to 6.12 mg kg<sup>-1</sup>, with maximum Fe content observed in  $T_7$ , followed by  $T_6$  (5.72 mg kg<sup>-1</sup>). These results are in close conformity with earlier studies reporting significant increases in DTPA-extractable Zn and Fe following inoculation with Zn- and Fe-solubilising bacteria (87), attributed mainly to organic acid secretion and rhizosphere acidification. Comparable enhancements in soil micronutrient availability under microbial consortia application, with 35–42 % and 28–33 % increases in DTPA-extractable Zn and Fe, respectively, have also been documented in pulse crops (80, 62). In addition, improvements in zinc availability in lentil soils following rhizobial inoculation have been reported, with Zn content increasing from 2.47 ppm before cultivation to 5.42 ppm in control soils and 4.36 ppm in inoculated soils (86). Similar positive responses of soil micronutrient availability under PGPR-based consortia in legume-based systems have also been documented (88). The increased Zn and Fe availability observed in the present study can be ascribed to microbial mechanisms such as solubilisation of insoluble mineral complexes through organic acid production, siderophore-mediated chelation and enhanced microbial activity in the rhizosphere, thereby confirming the effectiveness of ZnSB consortia in improving soil micronutrient status.

In the present investigation, lentil grain micronutrient content varied significantly among treatments, clearly reflecting the influence of ZnSB consortia. Grain zinc concentration ranged from 21.40 to 46.80 mg kg<sup>-1</sup>, while grain iron content varied from 62.50 to 102.30 mg kg<sup>-1</sup>. The lowest Zn and Fe contents were recorded in the uninoculated control ( $T_1$ ), whereas treatment  $T_7$  registered the maximum accumulation of both micronutrients, highlighting the effectiveness of ZnSB consortia in improving grain nutritional quality. These observations are in strong agreement with earlier reports showing wide variation in seed Fe and Zn contents, ranging from

30.42 to 79.06 mg kg<sup>-1</sup> and 23.36 to 57.19 mg kg<sup>-1</sup>, respectively, across different treatments and soil systems, with microbial consortia consistently producing the highest micronutrient enrichment (28). Similar trends were also reported for zinc assimilation in soybean seeds, where Zn content increased from 47.12 µg g<sup>-1</sup> in the uninoculated control to 48.10–57.34 µg g<sup>-1</sup> following inoculation with zinc-solubilizing *Bacillus* isolates (30), further supporting the potential of ZnSB in enhancing crop Zn uptake and effective grain biofortification.

## Conclusion

This study demonstrated that zinc-solubilising bacteria (ZnSB) isolated from lentil rhizosphere and root nodules possess strong zinc solubilisation ability along with multiple plant growth-promoting traits. Among the screened (rhizospheric (Rh) and nodule-associated (Nd)) isolates, Rh-ZnSB 38, Rh-ZnSB 60, Rh-ZnSB 90 and Nd-ZnSB 1 showed superior performance for zinc solubilisation, nutrient mobilisation, siderophore production, phytohormone synthesis and compatibility with nodule isolate. The developed microbial consortium exhibited a synergistic effect, significantly enhancing seed germination, plant growth, nodulation, yield attributes and grain Zn and iron (Fe) concentrations under pot culture conditions. In addition, consortium application improved soil fertility parameters, including available nitrogen-phosphorus-potassium (NPK), organic carbon and diethylenetriaminepentaacetic acid (DTPA)-extractable Zn and Fe. Overall, the ZnSB consortium represents a promising bioinoculant for sustainable lentil production and micronutrient biofortification, particularly in zinc-deficient soils.

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## Authors' contributions

KR and AJ conceptualised the experiment. KR performed the methodologies involved in this study. DP and GK reviewed, edited and supervised the experiment. HP, SMK and NCV carried out the statistical analysis and contributed to editing the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflicts of interests to declare.

**Ethical issues:** None

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