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Combining Indigenous Endophytes with Reduced NPK Fertilization Enhances Yield and Phytochemical Quality of Roselle (*Hibiscus sabdariffa* L.) in Arid Conditions

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Abstract

The intensive use of chemical fertilizers in medicinal plant production raises significant environmental and quality concerns, particularly under arid and high-temperature conditions. This study investigated the effectiveness of indigenous endophytic bacteria consortium as a sustainable approach to reduce mineral fertilizer inputs while improving the growth, yield, and phytochemical quality of roselle (*Hibiscus sabdariffa* L.) under Upper Egypt conditions. A field experiment was conducted during the summer of 2024 in Aswan, Egypt, using a factorial randomized complete block design. Treatments included a ten-strain endophytic consortium applied alone or combined with 25%, 50%, and 75% of the recommended NPK dose, alongside an unfertilized control and 100% NPK alone. Results highlighted clear percentage-based improvements with integrated treatments. The combination of 75% NPK with endophytic inoculation increased dry calyx yield by 16% relative to the conventional 100% NPK treatment. Significant increases were also observed in vegetative growth, fruit number, biomass accumulation, and photosynthetic pigments relative to full chemical fertilization. Moreover, antioxidant activity and concentrations of anthocyanins, phenolics, and flavonoids were maintained or enhanced under reduced fertilizer regimes, indicating qualitative gains without yield penalties. In contrast, complete fertilizer omission caused marked reduction in growth and yield parameters. Overall, substituting 25% of mineral fertilizers with indigenous endophytic inoculation not only sustained productivity but generated measurable yield gains, improved nutrient use efficiency, and strengthened crop resilience, demonstrating a practical and environmentally sound strategy for sustainable roselle cultivation in arid regions.

Keywords: *Hibiscus sabdariffa* L.; endophytic bacteria; nitrogen fertilization; plant growth promotion; sustainable agriculture; soil–plant–microbe interactions

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1. Introduction

Endophytic microorganisms are plant-associated microbes that inhabit internal tissues without causing disease. By colonizing the endosphere—a relatively stable and protected internal environment—these microbes form close, long-term associations with their host plants, and often display stronger functional integration than rhizospheric microorganisms [1–3]. Through both direct and indirect mechanisms, endophytes enhance plant growth and productivity by improving nutrient acquisition, increasing stress tolerance, modulating phytohormone balance, and stabilizing yield under unfavorable environmental conditions.

Internal plant tissues provide endophytes with access to carbohydrates, amino acids, vitamins, and minerals, supporting sustained microbial activity. Plant-derived substrates have therefore been used to culture diverse microbial communities, including beneficial and pathogenic fungi [4]. Numerous studies report that bacterial endophytes from genera such as *Pseudomonas*, *Bacillus*, *Azospirillum*, and *Rhizobium*, as well as fungal genera including *Trichoderma* and *Aspergillus*, enhance crop tolerance to heat and other abiotic stresses [5]. These plant-growth-promoting endophytes mitigate stress by stimulating antioxidant systems, improving nutrient solubilization and uptake, expanding root surface area, producing heat-shock proteins, triggering induced systemic resistance, and regulating phytohormones such as auxins, gibberellins, and abscisic acid [6–8].

Medicinal plants provide a valuable model for studying endophyte–plant interactions. Approximately 10% of vascular plant species have been used in traditional medicine since ancient times [9,10], and hundreds of species are currently cultivated worldwide, particularly in Asia and Africa, for pharmaceutical, nutraceutical, and herbal applications. According to the World Health Organization (WHO), nearly 90% of Member States report the use of traditional medicine, underscoring its global importance. The WHO Global Traditional Medicine Strategy 2025–2034 highlights the widespread practice of traditional medicine across all regions and emphasized the need for sustainable production systems that ensure safety, quality, and efficacy [11]. International standards therefore stress accurate botanical identification and strict quality control of medicinal plant products [12].

Egypt has a long tradition of cultivating medicinal and aromatic plants, which contribute significantly to the agricultural economy and export market. Although the country benefits from favorable climatic conditions, its share of global production remains modest at approximately 2.3%. Nevertheless, these crops represent about 5.72% of total agricultural exports, valued at approximately USD 184.3 million in 2021 [13]. Among these crops, roselle (*Hibiscus sabdariffa* L.) locally known as karkade, holds considerable economic and cultural significance. Native to Africa, roselle is cultivated widely in tropical and subtropical regions, with Egypt, Sudan, Mexico, Thailand, and China among the leading producers [14].

Roselle is an herbaceous shrub in the family Malvaceae, typically growing 1.5–2.0 m tall [15]. Its calyces, leaves, stems, and seeds are used for nutritional, medicinal, and industrial purposes. The calyces are particularly rich in organic acids (tartaric, oxalic, malic, ascorbic, and succinic acids), anthocyanins (delphinidin and cyanidin), phenolic compounds, vitamins, minerals, β-carotene, lycopene, polysaccharides, and pectin, contributing to strong antioxidant and antimicrobial activities [16,17]. Roselle seeds contain approximately 3.2% sterols, including ergosterol [18]. Anthocyanins also play a protective role in plants by enhancing tolerance to biotic and abiotic stresses [19].

In Egypt, Aswan Governorate represents the main production area for roselle, accounting for nearly 53% of the total cultivated area [20]. However, cultivation in this region faces multiple constraints. Soils are predominantly sandy to sandy loam, characterized by low organic matter, weak water-holding capacity, and limited nutrient retention [21]. These limitations are further aggravated by salinity issues [22,23] and, in some cases, heavy metal

accumulation [24]. Long-term dependence on chemical fertilizers has also contributed to soil degradation, reduced nutrient use efficiency, suppression of secondary metabolite synthesis, and increased vulnerability to pests and diseases [25].

Climatic stress compounds these problems. Summer temperatures in Aswan frequently exceed 45 °C, leading to oxidative stress caused by excessive reactive oxygen species (ROS) accumulation. This stress impairs photosynthesis, reduces biomass and yield, accelerates senescence, and may induce premature fruiting [26,27]. To compensate, farmers often apply high nitrogen inputs, given nitrogen's key role in vegetative growth and secondary metabolite production [28]. However, excessive nitrogen fertilization can result in soil contamination, chemical residues in medicinal products, and long-term environmental concerns [25]. Thus, conventional fertilization practices increasingly conflict with sustainable medicinal plant production, highlighting the need for environmentally friendly alternatives [29,30].

Exploiting the native endophytic microbiome of roselle represents a promising strategy to address these challenges. Indigenous endophytic bioinoculants may enhance plant resilience, productivity, and phytochemical stability while reducing reliance on chemical fertilizers. Therefore, this study evaluated selected roselle-derived endophytic bacteria as eco-friendly bioinoculum under the agro-climatic conditions of Aswan. Specifically, we examined the combined application of indigenous endophytes with reduced NPK fertilization to improve growth, yield, and secondary metabolite production, contributing to more sustainable roselle cultivation in arid and heat-stressed environments.

2. Materials and Methods

Based on the functional trait screening [31], a consortium composed of ten bacterial strains was selected and tested on *Hibiscus sabdariffa* under open-field conditions. The present study reported the field experiment results, emphasizing the impact of bacterial inoculation on vegetative growth, yield components, and quality-associated traits.

The experiment was carried out during the summer season 2024 at the experimental farm of the Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt (24°05′53″ N, 32°53′57.91″ E). The site is characterized by sandy soil and extremely high summer temperatures (Figure 1).

2.1. Isolation, Characterization, and Evaluation of Endophytic Bacterial Isolates

2.1.1. Isolation and Morphophysiological Characterization of Endophytic Bacteria from Roselle Roots

Endophytic bacteria associated with roselle roots were isolated using a modified surface-sterilization protocol. Root samples (13.05 g) were washed, dried, and sterilized sequentially with 75% ethanol and 5% sodium hypochlorite, followed by multiple rinses with sterile distilled water. Sterilization efficiency was confirmed by plating the final rinse on nutrient agar and incubating at 30 °C for 24 h. Sterilized roots were cut, crushed, and homogenized in 0.85% potassium chloride saline solution, and serial dilutions were prepared.

Diluted samples were cultured on nutrient agar and roselle agar plates and incubated at 30 °C for 24–72 h. Colonies from the 10^{-3} dilution were purified by repeated streaking and preserved in 70% glycerol at −20 °C. Representative plates containing 30–70 CFU were selected for morpho-physiological characterization, including colony morphology, cell morphology, Gram staining, and cultural features.

Figure 1. The experimental site located in the Experimental farm of Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt.

2.1.2. Screening of Plant Growth–Promoting Traits in Endophytic Bacterial Isolates

Endophytic bacterial isolates were screened for plant growth–promoting traits. Indole-related compound production was assessed using Glickmann and Dessaux's method [32]. Phosphate and potassium solubilization were evaluated on modified Pikovskaya's [33], and Aleksandrov media [34], respectively. Nitrogen fixation was determined by the micro-Kjeldahl method [35,36] and thermotolerance was tested under elevated temperature conditions [37,38].

2.1.3. Molecular Identification and Phylogenetic Analysis of Bacterial Isolates Using 16S rRNA Gene Sequencing

Bacterial isolates were identified through partial sequencing of the 16S rRNA gene. Genomic DNA was extracted using a commercial kit (ExoSAP-IT®, Seoul, Republic of Korea) following the manufacturer's protocol. The ~1500 bp 16S rRNA gene was amplified by PCR using universal primers 27F and 1492R in a 25 µL reaction mixture containing buffer, $MgCl_2$, dNTPs, primers, and Taq polymerase. Thermal cycling included initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. Purified amplicons were sequenced using the BigDye® Terminator v3.1 kit (Applied Biosystems, Foster city, CA, USA) on an ABI PRISM 3730XL Analyzer (Macrogen Inc., Seoul, Republic of Korea). Sequences were analyzed using NCBI BLAST (GenBank) and EzBioCloud, aligned in SnapGene, and phylogenetically evaluated by the neighbor-joining method in MEGA version 11.

2.1.4. Inoculum Formulation

Table 1 represented ten bacterial isolates exhibiting the highest plant growth–promoting rhizobacteria traits which were selected based on functional screening from the previous study [31] and Table 2 represented the molecular identification based on the 16S rRNA gene sequence in the database. The isolates were combined in equal proportions to prepare a composite inoculum at a final concentration of 5×10^6 CFU mL^{-1} in nutrient medium. Each isolate was cultured separately and gently agitated at 150 rpm for 1 h prior to mixing. Roselle seeds were soaked overnight in the composite inoculum (250 mL per isolate). The optical density of the resulting mixture was measured at 600 nm (OD_{600}) using a spectrophotometer (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany). In addition to seed inoculation, a booster dose of the bacterial inoculum was applied 45 days after planting at a rate of 10 mL per plant.

Table 1. The selected potent endophytes isolate recorded the highest values in plant-growth-promoting traits under in vitro conditions.

S. NO	Isolates Code	Indole Acetic Acid mg L^{-1}	N2 Assay mg L^{-1}	Solubilization of		Temperature Tolerance (°C)		
				P (PSI) *	K (KSI) **	45	50	55
1	HER15Z	11.13	32.67	2.21	2.61	+ve	+ve	−ve
2	HER21Z	16.50	-	2.29	2.68	+ve	+ve	−ve
3	HER43Z	25.85	30.80	2.43	2.31	+ve	+ve	−ve
4	HER36Z	37.01	-	2.35	2.58	+ve	+ve	−ve
5	HER46Z	26.34	35.47	2.61	2.30	+ve	+ve	−ve
6	HER12Z	5.83	28.93	2.30	2.26	+ve	+ve	−ve
7	HER25Z	24.46	29.87	-	-	+ve	−ve	−ve
8	HER28Z	30.86	-	2.27	2.25	+ve	−ve	−ve
9	HER23Z	30.08	-	-	2.39	+ve	−ve	−ve
10	HER15WZ	3.28	-	2.45	2.30	+ve	+ve	−ve

* PSI; Phosphate Solubilization Index; ** KSI; Potassium Solubilization Index; +ve: growth; −ve: absence of growth.

Table 2. The BLAST results, including the closest species of type strain, similarity percentage, and accession number of 10 isolates obtained from roselle roots.

Isolates Code	Closest Relative a	Acc. No	Id. (%)
1. HER15Z	*Bacillus sp.* A-BT-15	PX414331	99.2
2. HER21Z	*Bacillus cereus* strain JUB1	PX414332	100
3. HER43Z	*Bacillus sp.* B55	PX414334	99.64
4. HER36Z	*Ochrobactrum sp.* Strain QY-1	PX414333	100
5. HER46Z	*Achromobacter sp.* strain KR4-110	PX414335	99.28
6. HER12Z	*Neorhizobium sp.* CSC1952	PX414329	100
7. HER25Z	*Bacillus cereus* strain BE23	PX414336	99.14
8. HER28Z	*Bacillus paramycoides* strain karimi S.M1	PX414337	99.68
9. HER23Z	*Bacillus cereus* strain T0-10	PX414330	95.52
10. HER15WZ	-	ND	-

a: Based on the 16S rRNA gene sequence in the database. ND: not determined, as the sequence of this isolate was too short for database submission and getting accession number; accordingly, it is not included in the phylogenetic tree.

2.2. Plant Materials and Growth Condition

The local variety "Lotus-flowered" of red roselle, *Hibiscus sabdariffa* L., was used in this study. The seeds were obtained from the Horticulture Department, Faculty of Agriculture and Natural Resources, Aswan University, Egypt. On 1 June 2024, seeds were manually sown in well-prepared soil. Each subplot measured 5.0 × 1.2 m and consisted of two rows

spaced 30 cm apart. Approximately 30 days after sowing, seedlings were thinned to maintain two plants per hill, resulting in a total of 64 plants per subplot (32 plants per row). A drip irrigation system was installed to ensure uniform and efficient water supply. All other agronomic practices were preformed according to the standard recommendations throughout the growing season.

The soil at the experimental site was classified as sandy. Its physical and chemical properties were analyzed according to the procedures described by Jackson [35] and Black et al. [39], and the results are presented in Table 3.

Table 3. Some physical and chemical properties of soil used in isolating microbes.

Characteristics	Value
Physical properties	
Clay (%)	3.07
Silt (%)	0.00
Sandy (%)	96.50
Textural class	Sandy
Chemical properties	
pH	8.06
EC (dSm-1) at 25 °C	0.00
Soluble anions (meq/L)	
CO_3^-	0.00
HCO_3^-	7.02
Cl^-	3.53
SO_4^-	0.48
Soluble cations (meq/L)	
Ca^+	3.15
Mg^+	1.07
K^+	0.88
Na^+	0.84
Available macronutrients (ppm)	
Available N	131.00
Available P	10.00
Available K	180.00

2.3. Experimental Design

The experiment was arranged in a randomized complete block design (RCBD) in a factorial structure with four replicates. Both chemical fertilizer and a bacterial bio-formulation were evaluated. The recommended full chemical fertilizer dose (100% NPK) consisted of 250 kg ha^{-1} ammonia nitrate (33.5%), 150 kg ha^{-1} superphosphate (19.5%), and 75 kg ha^{-1} potassium sulphate (49.5%). The bio-formulation comprised the ten selected bacterial isolates described previously. Six treatments were tested as follow: (1) unfertilized control (no chemical fertilizer or bio-formulation), (2) bio-formula alone, (3) 25% NPK plus bio-formula, (4) 50% NPK plus bio-formula, (5) 75% NPK plus bio-formula, and (6) 100% NPK (recommended chemical fertilizer dose, positive control).

2.4. Data Recorded

2.4.1. Vegetative and Yield Characteristics

At 116 days after sowing (blooming stage), five representative plants per treatment were randomly sampled from each replicate to assess vegetative growth and yield parameters. The vegetative measurements included plant height (cm), stem diameter (mm),

number of branches, and shoot fresh weight (FW) and dry weight (DW). Yield-related traits comprised number of fruits per plant, fresh and dry weights of the calyxes and seeds (g plant^{-1}), and total dry calyx yield (kg ha^{-1}).

2.4.2. Chemical Characteristics

- Photosynthetic pigments

During the fruiting stage, fully expanded healthy leaves were randomly collected from the middle portion of plants in each treatment. Chlorophyll "a", chlorophyll "b", and carotenoid contents were determined according to Metzner et al. [40]. Leaf pigments were extracted in 80% (v/v) aqueous acetone. Absorbance was recorded at 663, 644 and 452.5 nm using a SPECTRO star Nano spectrophotometer (BMG LABTECH GmbH, Ortenberg, Germany). Pigment concentrations were calculated using the following equations:

Chlorophyll "a" = (10.3 × E663) − (0.918 × E644) (µg/mL)
Chlorophyll "b" = (19.7 × E644) − (3.87 × E663) (µg/mL)
Carotenoids = (4.2 × E452.5) − (0.0264 × Chlorophyll "a") − (0.426 × Chlorophyll "b") (µg/mL)

Values of chlorophyll "a", chlorophyll "b", and carotenoids were subsequently converted to µg g^{-1} fresh weight.

- Mineral content

For mineral analysis, 0.2 g of dried leaf material was digested using a 1:1 (v/v) mixture of concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) in a heating digestion system (DK, VELP Scientific Srl, Usmate Velate, Italy). The resulting digest was used for nutrient determination.

Nitrogen (%) was measured using the micro-kjeldahl [41].

Phosphorus (%) was determined colorimetrically using ammonium molybdate and ascorbic acid reagents, with absorbance measured by spectrophotometry using a SPECTRO star Nano (BMG LABTECH GmbH, Ortenberg, Germany) [42].

Potassium (%) was quantified using a flame photometer [41].

Calcium (%) was analyzed using an atomic absorption spectrophotometry following Pearson [43].

2.4.3. Bioactive Compounds Determination

- Total anthocyanin content

At harvest, total anthocyanins in roselle calyces were quantified using the differential pH method [44]. Calyxes were air-dried in the shade, ground into powder, and 1 g of sample was extracted in acidified 70% ethanol (1% HCl). The mixture was incubated at 4 °C in darkness for seven days, and then centrifuged at 6000 rpm for 15 min. The residue was re-extracted to ensure maximum recovery.

Aliquots of the extract (1 mL) were separately mixed with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). Absorbance was measured at 520 and 700 nm using a UV–Vis spectrophotometer (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany) within 20–50 min of sample preparation. Acidified ethanol served as the blank.

Total anthocyanin content was calculated as cyanidin-3-glucoside (Cyd-3-Glu) equivalents (mg g^{-1} DW) using the following equation:

$$Total\ anthocyanins = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

where

$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}$, MW = molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}), DF = dilution factor, l = path length of the cuvette (1 cm), and ε = molar extinction coefficient of cyanidin-3-O-β-D-glucoside (26,900 L mol^{-1} cm^{-1}).

Measurements were confirmed using a secondary UV–Vis spectrophotometer (Optizen Pop, Mecasys, Daejeon, Republic of Korea).

- Total phenolic content

Total phenolics were determined using the Folin–Ciocalteu method, with slight modifications [45]. Briefly, 0.5 g of dried calyx powder was extracted in 20 mL of distilled water. An aliquot (1 mL) was mixed with 5 mL of ethanol 70% and 100 µL of Folin–Ciocalteu reagent, and was allowed to react for 3 min, followed by addition of 150 µL of sodium carbonate (20%, *w*/*v*). After incubation for an hour at room temperature, absorbance was measured at 765 nm using a SPECTRO star Nano (BMG LABTECH GmbH, Ortenberg, Germany), and distilled water as blank. The results were expressed in mg gallic acid equivalents per g dry weight (mg GAE g^{-1} DW).

- Total flavonoids content

Flavonoids were quantified using aluminum chloride colorimetric assay [46]. An aliquot (1 mL) of the ethanolic extract was sequentially mixed with 150 µL sodium nitrate (1 mol/L), 150 µL of aluminum chloride (10%), and 1 mL of sodium hydroxide (1 mol/L) with vortexing for 3 min between each step. Absorbance was recorded at 510 nm and 650 nm against a reagent blank using SPECTRO star Nano (BMG LABTECH GmbH, Ortenberg, Germany). Flavonoids was expressed as mg catechin equivalents per 100 g dry weight (mg CE 100 g^{-1} DW).

- Antioxidant activity (DPPH assay)

Radical scavenging activity was assessed using the DPPH method [47] with minor adjustments. Dried calyx powder (1 g) was extracted in 40 mL of 70% ethanol and diluted appropriately (1:5). The sample extract (1 mL) was mixed with 4 mL of 95% ethanol solution and DPPH-ethanol mixed solution (2 mL, 0.05 mg·mL^{-1}), and incubated for 30 min in the dark. Absorbance was measured at 517 nm using SPECTRO star Nano (BMG LABTECH GmbH, Ortenberg, Germany). DPPH scavenging activity (%) was calculated as:

$$DPPH\ (\%) = (A1 - A2)/A0 \times 100$$

where A0 is the control absorbance, A1 is the sample absorbance, and A2 is the blank absorbance.

2.5. Statistical Analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) according to Snedecor and Cochran [48]. Treatment means were compared using the Least Significant Difference (L.S.D.) test as described by Gomez and Gomez [49]. Statistical computations were performed using R software (version 2023, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Vegetative Growth and Yield-Related Traits

One-way ANOVA indicated that fertilization strategy significantly affected vegetative growth and yield-related traits of roselle (Table 4). Plants grown without fertilizer consistently showed the lowest performance across all parameters, highlighting the essential role of nutrient supply. In contrast, integrating bioinoculum with reduced rates of mineral NPK generally enhanced plant growth, fruit production, and yield efficiency compared with the full (100%) chemical fertilization regime.

Table 4. Effect (*F*-value) of different levels of chemical fertilizer with bioinoculum on plant height, stem diameter, number of branches, shoot fresh and dry weight, number of fruits as well as dry calyxes and seeds weight per plant and yield per hectare of roselle.

Variable	*F* Value	Probability	Variable	*F* Value	Probability
Vegetative growth traits			Yield-related traits		
Plant height	18.78	<0.0001 ***	Number of fruits	9.18	0.0009 ***
Stem diameter	8.35	0.0013 ***	Calyxes dry weight	10.54	0.0005 ***
Number of branches	5.13	0.0096 ***	Calyxes dry yield	10.54	0.0005 ***
Shoot fresh weight	9.35	0.0008 ***	Seeds dry weight	9.81	0.0006 ***
Shoot dry weight	93.91	<0.0001 ***	Seeds dry yield	9.81	0.0006 ***

*** represent significant at probability < 0.01.

Vegetative growth parameters were markedly suppressed under the unfertilized treatment. However, combined bioinoculum with reduced NPK levels produced plant heights comparable to, or exceeding, those obtained with the recommended 100% NPK dose. In general, the most vigorous growth was observed under the 50% NPK plus bioinoculum treatment (Figure 2), suggesting that indigenous endophytes effectively compensated for lower mineral fertilizer inputs and promoted greater biomass accumulation.

Stem diameter reached its maximum under the full 100% NPK treatment, showing a 23% increase over the control. In contrast, the 50% NPK combined with biofertilizer produced the tallest plants, with an 18% increase compared to the unfertilized treatment, along with values similar to or slightly higher than those recorded under 100% NPK. This treatment also resulted in the greatest number of branches, exceeding the control by 25% and surpassing the full NPK treatment by approximately 14%. The highest fresh and dry shoot weights were obtained with 25% NPK supplemented with bioinoculum. Fresh weight increased by 34% relative to the control and by 15% compared with the 100% NPK treatment. A similar pattern was observed for dry weight, which increased by 40% over the control and by 25% over the full NPK treatment.

Fruit number increased significantly when a portion of chemical fertilizer was replaced with indigenous endophytes consortium. Among fertilized treatments, plants receiving the full 100% NPK dose produced the lowest fruits number, whereas gradual substitution with bioinoculum resulted in a progressive increase, reaching a maximum under the 25% NPK plus bioinoculum treatment. As expected, unfertilized plants exhibited a pronounced reduction in fruit number (Figure 3).

Calyx fresh weight was highest under the recommended 100% NPK treatment. However, partial substitution of 25–75% of the mineral fertilizer with bioinoculum maintained fresh calyx weight at levels statistically comparable to the full NPK treatment. Significant declines were observed only in the bioinoculum-alone and fertilizer-free conditions (Figure 3).

Calyx dry weight and total yield responded positively to integrated nutrient management. The 75% NPK plus bioformula treatment produced the highest dry calyx weight and yield per hectare, surpassing the full chemical fertilization regime and demonstrating the effectiveness of combining bioinoculum with moderate mineral inputs. Further reduction in chemical fertilizers beyond this level led to significant yield losses, particularly under bioinoculum-only and unfertilized treatments. Although the 50% NPK plus bioinoculum treatment promoted the greatest vegetative growth, the 75% NPK plus formula treatment appeared to optimize assimilate partitioning toward reproductive organs (calyces), resulting in the highest economic yield. This outcome suggests an improved source–sink balance rather than merely increased biomass production.

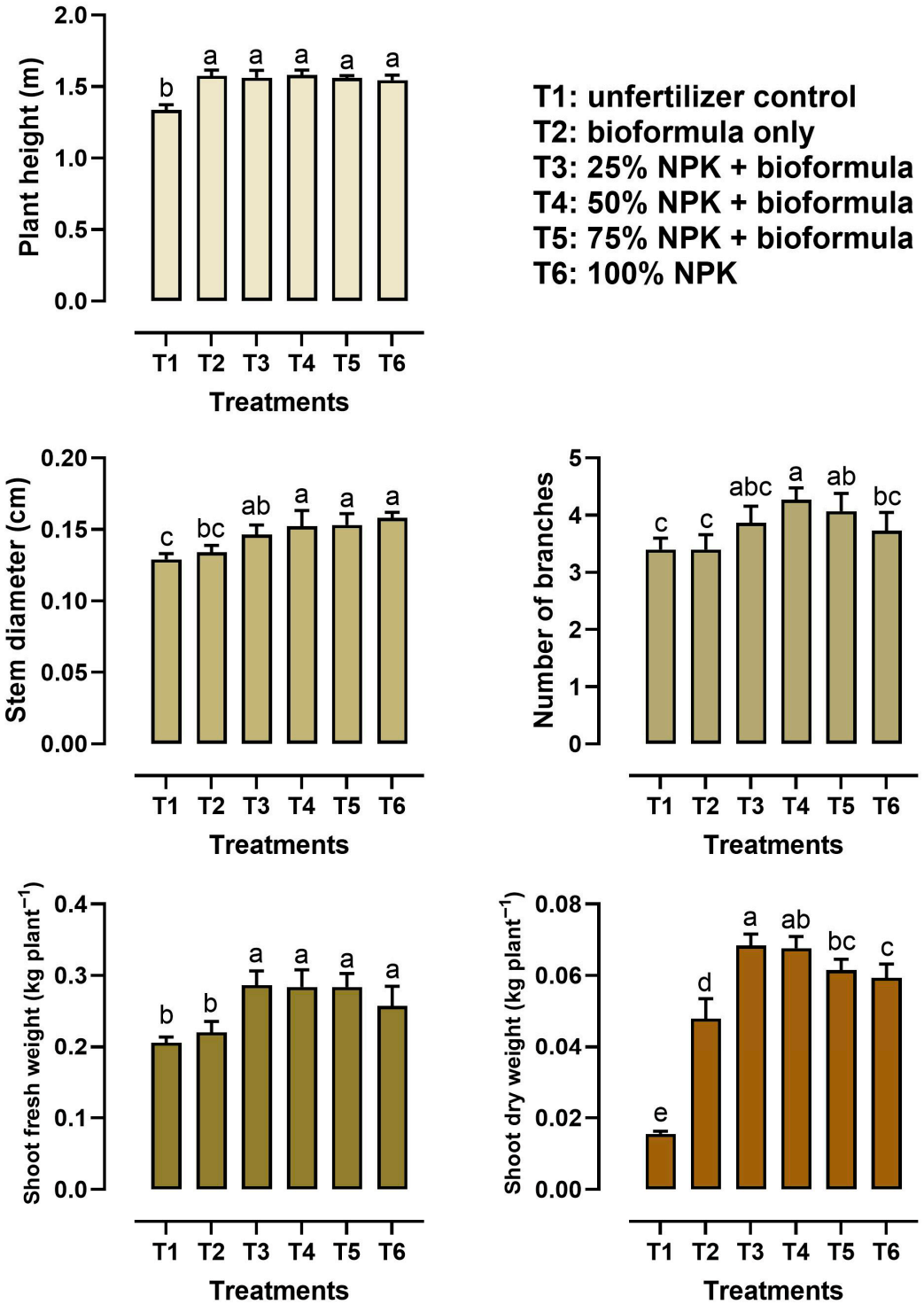

Figure 2. Effect of different levels of chemical fertilizer with bioinoculum on plant height, stem diameter, number of branches as well as roselle shoot fresh and dry weight. Different letters indicate significant differences between treatment (oe-way ANOVA, $p \leq 0.05$).

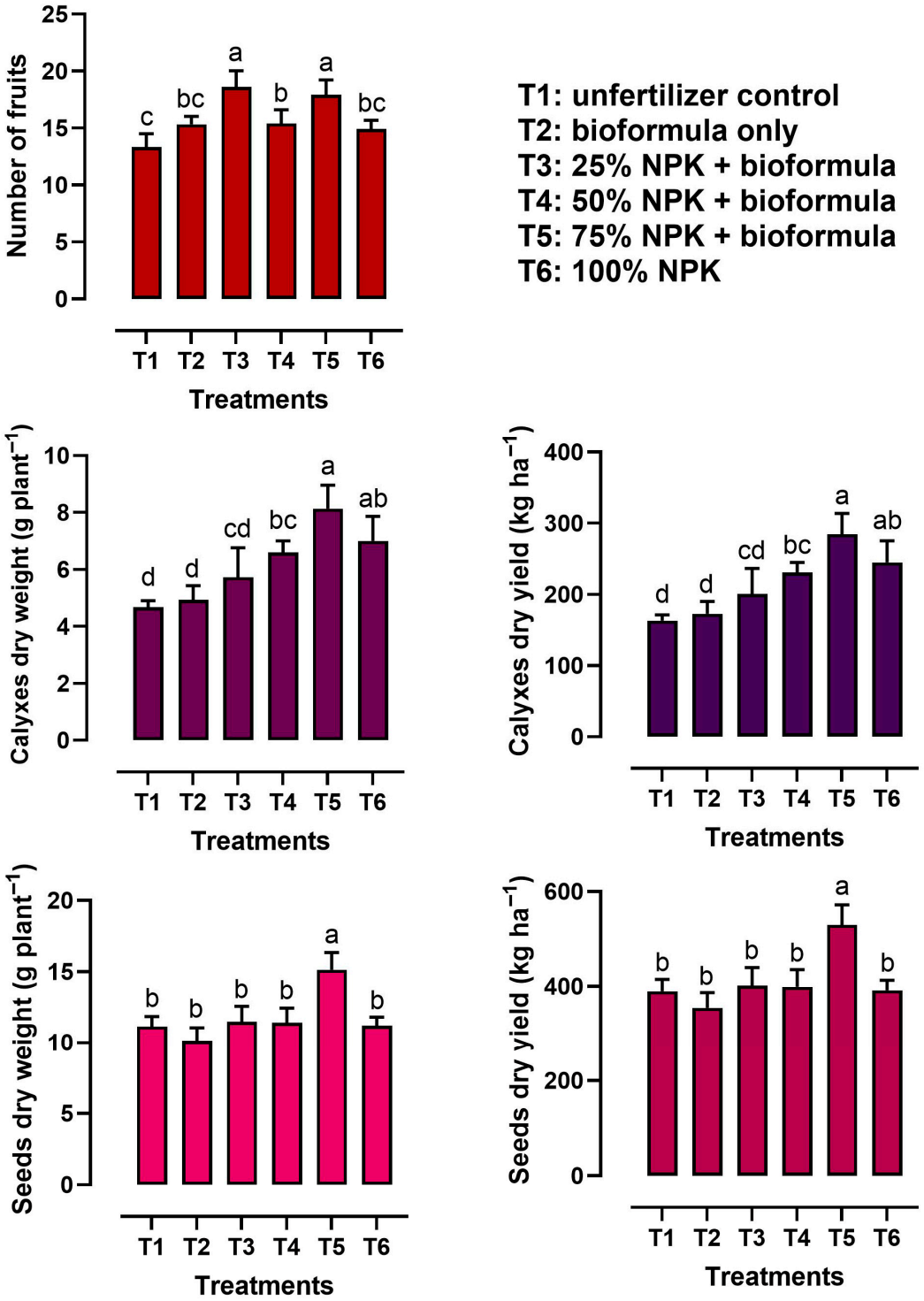

Figure 3. Effect of different levels of chemical fertilizer with bioinoculum on number of fruits as well as dry calyxes and seeds weight per plant and yield per hectare of roselle. Different letters indicate significant differences between treatment (oe-way ANOVA, $p \leq 0.05$).

Flowering data further support this trend: the 25% NPK combined with bioinoculum treatment produced the greatest number of flowers, increasing by 40% compared to the unfertilized control and by 25% relative to the full 100% NPK treatment. In contrast, calyx weight, total calyx yield, seed weight, and seed yield were maximized under the 75% NPK plus bioinoculum treatment, which exceed the control by 74% for calyx yield and by 36% for seed yield, and outperformed the 100% NPK treatment by 16% and 35%, respectively.

These findings demonstrate that integrating indigenous endophyted consortium with reduced chemical fertilization enhances reproductive performance, improves yield components, and increases production efficiency in roselle while reducing dependence on full mineral NPK inputs.

3.2. Chemical Analysis

3.2.1. Photosynthetic Pigments

One-way ANOVA indicated that fertilizer treatments had no significant effects on chlorophyll a or chlorophyll b contents (F = 1.58 and 0.39; p = 0.24 and 0.84, respectively). In contrast, carotenoids content was significantly influenced by fertilization strategy (F = 8.98; p = 0.001) (Table 5). Although chlorophyll differences were not statistically significant, the highest chlorophyll a value was recorded under the 75% NPK combined with bioinoculum treatment, representing increases of 25% over the untreated control and 15% over the full (100%) NPK dose. Chlorophyll b content remained relatively stable across all treatments.

Table 5. Effect (F-value) of different levels of chemical fertilizer with bioinoculum on photosynthetic pigments and some minerals (N, P, K and Ca %) of roselle leaves as well as bioactive compounds (antioxidants, phenolics, flavonoids and anthocyanin) of roselle calyxes.

Variable	*F* Value	Probability	Variable	*F* Value	Probability
Chlorophyll a	1.59	0.24 ^{ns}	Calcium (Ca%)	36.19	<0.0001 ***
Chlorophyll b	0.39	0.84 ^{ns}	Total Antioxidants	6.34	0.0042 ***
Carotenoids	10.53	0.0005 ***	Total Phenolic	0.41	0.83 ^{ns}
Nitrogen (N%)	2.29	0.11 ^{ns}	Total Flavonoids	0.37	0.86 ^{ns}
Phosphorus (P%)	2.75	0.07 ^{ns}	Total Anthocyanins	0.87	0.53 ^{ns}
Potassium (K%)	8.60	0.0012 ***			

*** represent significant at probability < 0.01; ^{ns} represent not significant differences.

Carotenoid concentration was maximized under the 25% NPK plus bioinoculum treatment, increasing by 84% compared with the control and by 21% relative to the 100% NPK treatment. A gradual, non-significant decline in carotenoid levels was observed with increasing mineral fertilizer rates. The lowest carotenoid value (0.55 µg g^{-1}) was recorded in unfertilized plants (Figure 4).

The elevated carotenoid content under reduced NPK combined with bioinoculum may reflect a mild nutrient-stress stimulus that activates photoprotective and antioxidant defense mechanisms. This response, potentially enhanced by endophytic activity, appears to promote stress adaptation without imposing substantial growth penalties, suggesting that carotenoid accumulation represents a regulated adaptive response under moderate nutrient limitation.

3.2.2. Mineral (N-P-K) Content

One-way ANOVA indicated that fertilizer treatments had no significant effects on nitrogen or phosphorus content (Table 5), suggesting that partial substitution of mineral fertilizer with bio-formula did not alter plant N or P status. In contrast, potassium and calcium concentrations were significantly influenced by fertilizer strategy.

Potassium content declined progressively as mineral NPK levels were reduced. The highest potassium concentration (1.24%) was recorded under the full 100% NPK treatment, whereas the lowest value (0.84%) was observed in unfertilized plants. A similar pattern was observed for calcium, which showed highly significant differences among treatments (F = 36.19; p < 0.0001). Calcium concentration reached 2.95% under the full NPK dose, decreased to 2.11% with bioinoculum alone, and dropped further to 1.15% in the fertilizer-free treatment (Figure 5).

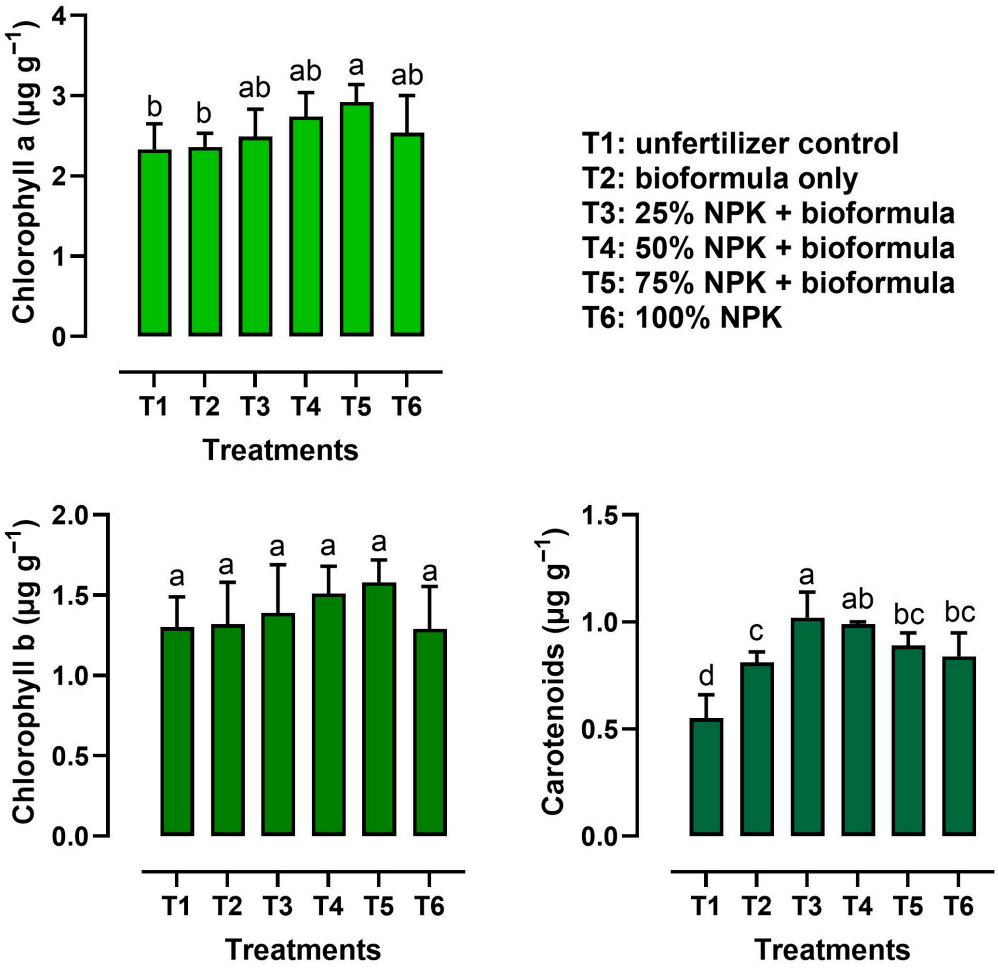

Figure 4. Effect of different levels of chemical fertilizer with bioinoculum on photosynthetic pigments in roselle leaves. Different letters indicate significant differences between treatment (oe-way ANOVA, $p \leq 0.05$).

The recommended 100% NPK treatment produced the highest mineral content for all measured elements. Compared with the untreated control, this treatment increased nitrogen by 9%, phosphorus by 36%, potassium by 48%, and calcium by 100%.

3.2.3. Bioactive Compound

Total phenolic compounds, flavonoids, and anthocyanins were not significantly affected by the partial replacement of chemical fertilizer with bio-formula. In contrast, antioxidant activity showed significant variation among treatments (Table 5). The highest antioxidant activity was recorded in plants treated with 75% NPK plus bioinoculum treatment, while the lowest activity occurred under the 25% NPK plus bioinoculum treatment (Figure 6).

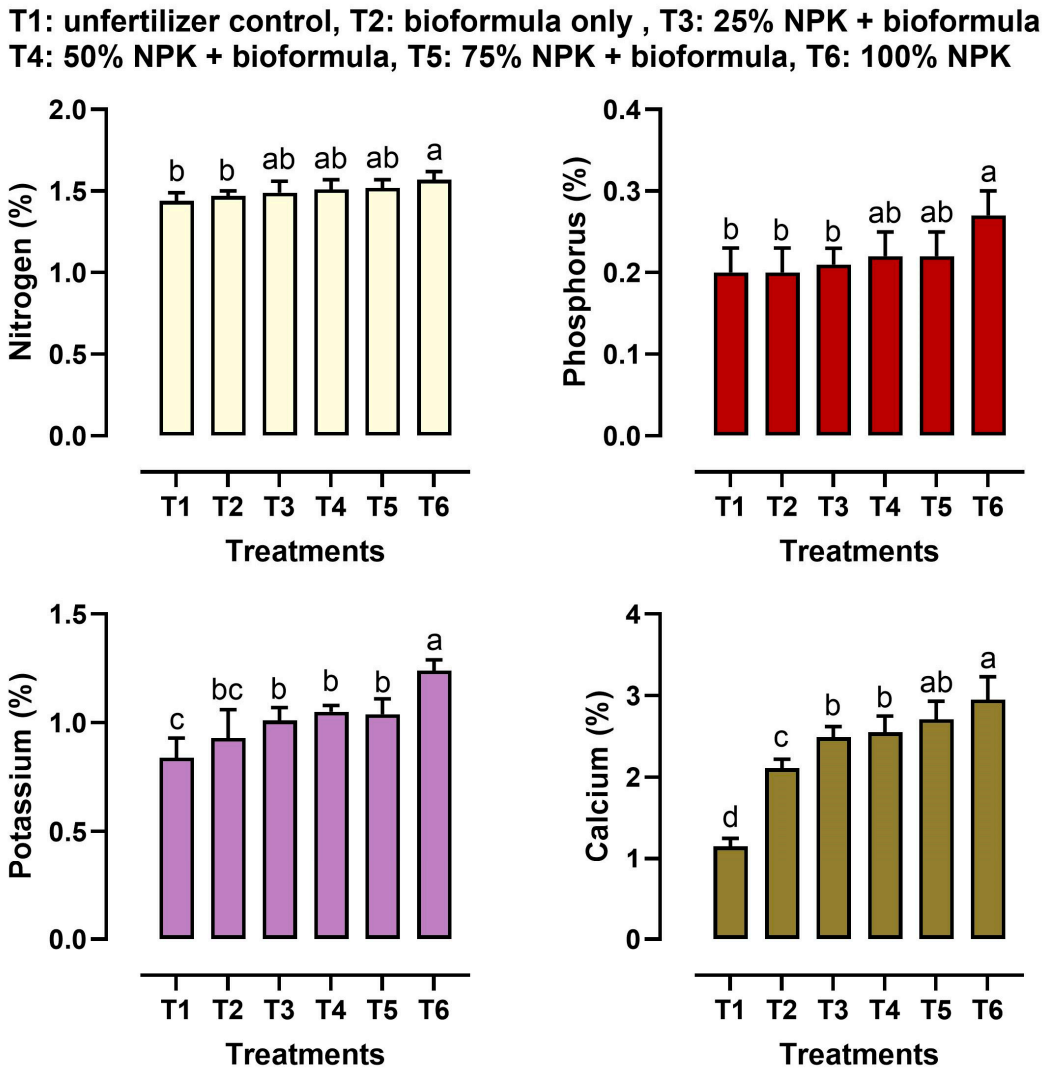

Figure 5. Effect of different levels of chemical fertilizer with bioinoculum on some minerals (N, P, K and Ca % of roselle leaves). Different letters indicate significant differences between treatment (oe-way ANOVA, $p \leq 0.05$).

Phenolic content and anthocyanin levels were highest in the untreated control and in plants receiving bioinoculum alone, whereas their lowest values were observed under the full 100% NPK treatment. However, differences in anthocyanins were not statistically significant. Flavonoid content reached its maximum under the 50% NPK plus bioinoculum treatment, showing a 16% increase relative to the control and values similar to or slightly higher than those obtained with 100% NPK.

These results indicate that partial substitution of mineral fertilizer with indigenous endophyted consortium can enhance antioxidant activity and flavonoid accumulation without significantly affecting total phenolics or anthocyanins.

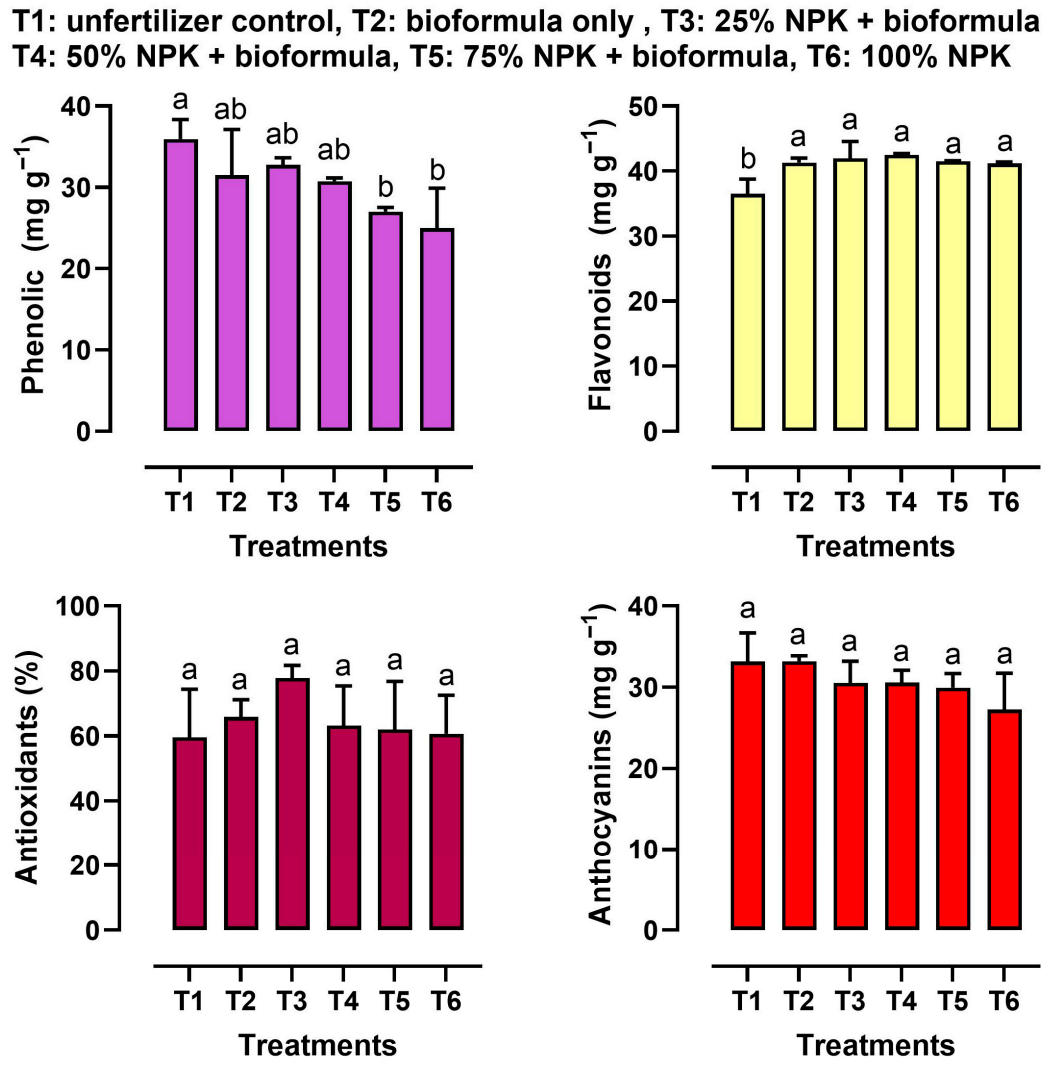

Figure 6. Effect of different levels of chemical fertilizer with bioinoculum on bioactive compounds (antioxidants, phenolics, flavonoids and anthocyanin of roselle calyxes). Different letters indicate significant differences between treatment (oe-way ANOVA, $p \leq 0.05$).

4. Discussion

Endophytic bacteria isolated from roselle roots have demonstrated strong potential as plant growth-promoting agents, exhibiting multiple functional traits such as phytohormone production, phosphate and potassium solubilization, nitrogen fixation, and thermotolerance. These traits are important for enhancing plant resilience in arid and nutrient-poor soils [31]. The present field experiment provided evidence that indigenous endophytic bacterial consortia can serve as effective bioinoculum, reducing dependence on chemical fertilizers while improving plant growth, nutrient uptake, and tolerance to abiotic stresses. These findings algin with Berg et al. [50], who highlighted the role of species- and habitat-specific plant microbiota in regulating nutrient acquisition, stress tolerance, seed germination, and overall holobiont functioning. Similarly, Oukala et al. [51] emphasized the influence of soil microbiota on plant physiology, with bacterial endophytes playing a central role in promoting plant health.

The potent isolates in this study, HER36Z, HER46Z, and HER21Z, expressed multiple plant growth-promoting (PGPR) traits, including high temperature tolerance (up to 50 °C), substantial indole-3-acetic acid (IAA) production, nitrogen fixation, and effective phosphorus and potassium solubilization. These attributes suggest that selected indigenous endophytes can form a robust consortium capable of enhancing roselle growth and

nutrient acquisition under stress conditions. These results are consistent with previous findings in roselle [52,53], *Aloe vera* [54], *Lolium perenne* [55], wild pistachio trees [56], and *Camellia sinensis* [57–59].

Integration of these bioinoculum enhanced the bioavailability of essential nutrients through nitrogen fixation and phosphorus/potassium solubilization, contributing to improved nutrient cycling and reducing reliance on chemical fertilizers. Experimental data showed that partial substitution of chemical fertilizers with bioinoculum supported vegetative biomass and yield. Notably, the 25% NPK plus bioinoculum treatment achieved shoot fresh weight comparable to the full 100% NPK treatment, demonstrating that strategic application of bioinoculum can maintain growth while minimizing chemical inputs. These observations are in agreement with studies on roselle [60], *Origanum syriacum* subsp. Sinaicum [61], and *Ocimum basilicum* [62].

Abiotic stresses, particularly high temperatures, are major constrains to crop productivity [27]. The thermal tolerance of the selected endophytic isolates offers a key advantage, enabling plants inoculated with these microbes to maintain growth and yield under challenging environmental conditions. These endophytes likely enhance plant resilience through hormone regulation, nutrient mobilization, and improved stress tolerance.

Moreover, the potential of endophytic microbiomes for producing exosomes plays a pivotal role in plant–microbe interactions, contributing to stress adaptation and enhanced plant resilience to abiotic challenges. Given that the identified endophytic microbiomes demonstrate high-temperature tolerance and multiple PGPR traits, it is plausible that they also produce exosomes that facilitate plant defense mechanisms and stress adaptation. These results are in line with Wu et al. [7] who showed better understanding of endophyte bacteria regarding its use with medicinal plants to increase yield and resilience to a range of environmental stressors [63] on *Solanum lycopersicum* L.

Among the tested fertilization regimes, the 75% NPK plus bioinoculum treatment emerged as the most effective in optimizing both vegetative and reproductive performance. While the 50% NPK plus bioinoculum treatment maximized vegetative growth, the 75% NPK plus bioinoculum treatment appeared to optimize assimilate partitioning toward reproductive organs, resulting in the highest calyx dry weight and total yield. Specifically, this treatment increased calyx dry weight and overall yield by 16% compared to full NPK application, demonstrating that moderate chemical fertilization combing with endophytes can enhance productivity while reducing environmental impacts. These results corroborate prior observations on plant height, number of branches, number of fruits, and calyx yield in roselle and other crops [64–66].

This study highlights the importance of integrating biological and mineral inputs to achieve sustainable crop management, particularly in arid and low-fertility soils. Reduced NPK combined with endophytic consortium maintained plant growth and phytochemical quality while improving yield efficiency. These findings support the development of alternative nutrition systems based on organic and biological sources, as demonstrated in *Ocimum basilicum* [67], and *Coriandrum sativum* [68], where endophytic bacterium enhanced growth, biomass accumulation, and overall productivity.

For future research, emphasis should be placed on sustainable crop management practices in nutrient-poor soils, including integration of biofertilizers, green manures, and reduced chemical fertilizer inputs to enhance soil fertility and support medicinal plant cultivation. Standardization and potential commercial development of rhizobacterial inoculants should also be pursued. Additionally, the inhibitory effects of high chemical fertilizer doses on beneficial microbial activity should be investigated to optimize the synergistic use of chemical and biological inputs for sustainable agricultural systems.

5. Conclusions

Endophytic microorganisms inhabit internal plant tissues without causing harm and enhance plant performance through improved nutrient acquisition, phytohormone production, stress tolerance, and yield promotion. However, their cultivation under laboratory conditions is often difficult, prompting the use of plant-based substrates that better reflect their native environments. Medicinal plants such as roselle (*Hibiscus sabdariffa* L.) are valuable reservoirs of beneficial endophytes due to their bioactive richness and economic importance.

In arid regions like Aswan, Egypt, roselle cultivation is constrained by poor soil fertility, salinity, nutrient imbalances, extreme heat, and heavy reliance on chemical fertilizers, which contribute to soil degradation and reduced microbial diversity. These challenges highlight the need for sustainable production strategies.

This study demonstrated that integrating selected indigenous endophytes inoculation with reduced NPK fertilization enhances roselle growth, yield, and quality under harsh field conditions. Substituting 75% of the recommended NPK dose with bio-inoculation improved fruit number, biomass, calyx dry weight, total yield, and seed weight compared with full chemical fertilization, while 50% NPK plus bio-inoculation showed the most favorable effects on quality traits.

In conclusion, partial replacement of chemical fertilizers with endophytic bioinoculants offers an effective, climate-resilient, and environmentally sustainable strategy for roselle production in arid and semi-arid regions. This integrated approach reduces chemical dependency, supports soil health, and maintains high productivity, contributing to long-term agricultural sustainability.

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References

1. Kobayashi, D.Y.; Palumbo, J.D. Bacterial endophytes and their effects on plants and uses in agriculture. In *Microbial Endophytes*; Bacon, C.W., White, J.F., Eds.; CRC Press: Boca Raton, FL, USA, 2000; pp. 213–250.
2. Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. Natural products from endophytic microorganisms. *J. Nat. Prod.* **2004**, *67*, 257–268. [CrossRef]
3. Senthilkumar, M.; Anandham, R.; Madhaiyan, M.; Venkateswaran, V.; Sa, T. Endophytic bacteria: Perspectives and applications. In *Bacteria in Agrobiology*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 61–96.
4. Osman, Z.A.; Elsanousi, S.M.; Elsheikh, E.A.E. Plant materials as probable growth promoters for certain fungi. *Eur. J. Exp. Biol.* **2012**, *2*, 1785–1791.
5. Khan, A.L.; Waqas, M.; Kang, S.M.; Hamayun, M.; Lee, I.J. Plant growth-promoting endophytic fungi: A sustainable approach for enhancing stress tolerance in crop plants. *J. Microbiol. Biotechnol.* **2016**, *29*, 123–135.

6. Sanayei, S.; Barmaki, M.; Ebadi, A.; Torabi-Giglou, M. Amelioration of water deficiency stress in roselle by AMF and PGPR. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2021**, *49*, 11987. [CrossRef]
7. Wu, W.; Chen, W.; Liu, S.; Wu, J.; Zhu, Y.; Qin, L.; Zhu, B. Beneficial relationships between endophytic bacteria and medicinal plants. *Front. Plant Sci.* **2021**, *12*, 646146. [CrossRef]
8. Kumar, R.; Swapnil, P.; Meena, M.; Selpair, S.; Yadav, B.G. Plant growth-promoting rhizobacteria (PGPR): Approaches to alleviate abiotic stresses for enhancement of medicinal plants. *Sustainability* **2022**, *14*, 15514. [CrossRef]
9. Salmerón-Manzano, E.; Garrido-Cardenas, J.A.; Manzano-Agugliaro, F. Worldwide research trends on medicinal plants. *Int. J. Environ. Res. Public Health* **2020**, *17*, 3376. [CrossRef]
10. Nath, R.; Kityania, S.; Nath, D.; Das Talukdar, A.; Sarma, G. An extensive review on medicinal plants with special reference to economic importance. *Asian J. Pharm. Clin. Res.* **2023**, *16*, 6–11. [CrossRef]
11. WHO. *Traditional Medicine*; World Health Organization: Geneva, Switzerland, 2025.
12. Palhares, R.M.; Drummond, M.G.; Brasil, B.S.A.F.; Cosenza, G.P.; Brandão, M.G.L.; Oliveira, G. Medicinal plants recommended by the WHO: DNA barcode identification associated with chemical analyses. *PLoS ONE* **2015**, *10*, e0127866. [CrossRef]
13. Ahmed, A.E.; El-Beltagy, A.E.; Farrag, A.A. Analytical study of the competitiveness of Egyptian medicinal and aromatic plants in the world markets. *J. Am. Sci.* **2024**, *20*, 61–69.
14. FAO. *FAOSTAT Database*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2018.
15. Dhar, P.; Kar, C.S.; Ojha, D.; Pandey, S.K.; Mitra, J. Chemistry, phytotechnology, pharmacology and nutraceutical functions of kenaf (*Hibiscus cannabinus* L.) and roselle (*Hibiscus sabdariffa* L.) seed oil: An overview. *Ind. Crops Prod.* **2015**, *77*, 323–332. [CrossRef]
16. Fallahi, H.R.; Ghorbany, M.; Aghhavani-Shajari, M.; Samadzadeh, A.; Asadian, A.H. Qualitative response of roselle to planting methods, humic acid application, mycorrhizal inoculation and irrigation management. *J. Crop Improv.* **2017**, *31*, 192–208. [CrossRef]
17. Mosaad Gaafar, D.E.S.; Baka, Z.A.M.; Abou-Dobara, M.I.; Shehata, H.S.; El-Tapey, H.M.A. Microbial impact on growth and yield of *Hibiscus sabdariffa* L. and sandy soil fertility. *Egypt. J. Soil Sci.* **2021**, *61*, 259–274. [CrossRef]
18. Hashem, H.A.E.A.; El-Hadidy, A.E.; Ali, E.A. Impact of some safe agricultural treatments on insect pests, vascular wilt disease management and roselle (*Hibiscus sabdariffa* L.) productivity under Siwa Oasis conditions. *Int. J. Environ.* **2017**, *6*, 139–162.
19. Middleton, E.; Kandaswami, C.; Harborne, J.B. *The Flavonoids: Advances in Research Since 1986*; Chapman & Hall/CRC: New York, NY, USA, 1993.
20. CAPMAS. *Central Agency for Public Mobilization and Statistics*; CAPMAS: Cairo, Egypt, 2023.
21. Gameh, M.A.; Abdalazem, A.H.; Khozyem, H.M.; Mohamed, A.G. Assessment of some physical and chemical properties of soils in West Edfu area, Aswan Governorate, Egypt. *Assiut J. Agric. Sci.* **2020**, *51*, 150–170. [CrossRef]
22. Karthikeyan, B.; Joe, M.M.; Islam, M.R.; Sa, T. ACC deaminase-containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus*. *Symbiosis* **2012**, *56*, 77–86. [CrossRef]
23. Qin, S.; Feng, W.W.; Zhang, Y.J.; Wang, T.T.; Xiong, Y.W.; Xing, K. Diversity of bacterial microbiota of coastal halophyte *Limonium sinense*. *Appl. Environ. Microbiol.* **2018**, *84*, e01533-18. [CrossRef] [PubMed]
24. Govarthanan, M.; Mythili, R.; Selvankumar, T.; Kamala-Kannan, S.; Rajasekar, A.; Chang, Y.C. Bioremediation of heavy metals using an endophytic bacterium *Paenibacillus* sp. RM isolated from the roots of *Tridax procumbens*. *3 Biotech* **2016**, *6*, 242. [CrossRef]
25. Liao, J.; Xia, P. Continuous cropping obstacles of medicinal plants: Focus on plant–soil–microbe interactions. *Sci. Hortic.* **2024**, *328*, 112927. [CrossRef]
26. Soliman, W.S.; Fujimori, M.; Tase, K.; Sugiyama, S. Oxidative stress under prolonged heat stress in *Lolium perenne*. *Grassl. Sci.* **2011**, *57*, 101–106. [CrossRef]
27. Soliman, W.S.; Fujimori, M.; Tase, K.; Sugiyama, S. Heat tolerance in *Lolium perenne* cultivars. *Environ. Exp. Bot.* **2012**, *78*, 10–17. [CrossRef]
28. Strzemski, M.; Dzida, K.; Dresler, S.; Sowa, I.; Kurzepa, J.; Szymczak, G.; Wójciak, M. Nitrogen fertilisation decreases bioactive compounds in *Carlina acaulis*. *Ind. Crops Prod.* **2021**, *170*, 113698. [CrossRef]
29. Pérez-Jaramillo, J.E.; Mendes, R.; Raaijmakers, J.M. Impact of plant domestication on rhizosphere microbiome assembly. *Plant Mol. Biol.* **2016**, *90*, 635–644. [CrossRef]
30. Machiani, A.; Javanmard, A.; Ostadi, A.; Alizadeh, K. Improvement in essential oil quantity and quality of thyme (*Thymus vulgaris* L.) by integrative application of chitosan nanoparticles and arbuscular mycorrhizal fungi under water stress conditions. *Plants* **2023**, *12*, 1422. [CrossRef] [PubMed]
31. Ibrahim, Z.A.; Soliman, W.S.; Konsowa, A.O.; Abbas, M.T. Bacterial endophytes from *Hibiscus sabdariffa* roots as growth-promoting candidates. *Aswan Univ. J. Sci. Technol.* **2025**, *5*, 55–69. [CrossRef]
32. Glickmann, E.; Dessaux, Y. A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl. Environ. Microbiol.* **1995**, *61*, 793–796. [CrossRef] [PubMed]

33. Pikovskaya, R.I. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* **1948**, *17*, 362–370.
34. Parmar, P.; Sindhu, S.S. Potassium solubilization by rhizosphere bacteria: Influence of nutritional and environmental conditions. *J. Microbiol. Res.* **2013**, *3*, 25–31.
35. Jackson, M.L. *Soil Chemical Analysis*; Prentice Hall of India: New Delhi, India, 1973.
36. Hegazi, N.A.; Hamza, A.M.; Othman, A.; Ali, S.; Sedik, M.Z.; Fayez, M. A modified combined carbon N-deficient medium for isolation, enumeration and mass production of diazotrophs. In *Nitrogen Fixation with Non-Legumes*; Malik, K.A., Mirza, M.S., Ladha, J.K., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1998; pp. 247–253. [CrossRef]
37. Khan, U.; Rahman, S.M.; Khan, S.; Roy, S.; Hossain, K.M. Effects of probiotics on productive performances and serum lipid profile of broiler as substitute of antibiotics. *Sci. Prog.* **2024**, *107*, 00368504241276259. [CrossRef]
38. Saidan, S.A.; Jarboui, R.; Alsharari, S.S.; Azab, M.S. Characterization and identification of thermophilic bacteria isolated from different sites located in Al-Jouf Region, Saudi Arabia. *J. Pure Appl. Microbiol.* **2024**, *18*, 1. [CrossRef]
39. Black, C.A.; Evans, D.O.; Ensminger, L.E.; White, J.L.; Clark, F.E.; Dinauer, R.C. *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*, 2nd ed.; Soil Science Society of America: Madison, WI, USA, 1982.
40. Metzner, H.; Rau, H.; Senger, H. Studies on synchronization of some pigment-deficient Chlorella mutants. *Planta* **1965**, *65*, 186–194. [CrossRef]
41. Chapman, H.D.; Pratt, P.F. *Methods of Soil, Plant and Water Analysis*; University of California, Division of Agricultural Sciences: Berkeley, CA, USA, 1961.
42. John, M.K. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Sci.* **1970**, *109*, 214–220. [CrossRef]
43. Pearson, D. *The Chemical Analysis of Foods*, 7th ed.; Churchill Livingstone: Edinburgh, UK, 1976.
44. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content by the pH differential method. *J. AOAC Int.* **2005**, *88*, 1269–1278. [CrossRef]
45. González, E.; Gómez-Caravaca, A.M.; Giménez, B.; Cebrián, R.; Maqueda, M.; Martínez-Férez, A.; Robert, P. Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during in vitro gastrointestinal digestion. *Food Chem.* **2019**, *279*, 40–48. [CrossRef]
46. Shraim, A.M.; Ahmed, T.A.; Rahman, M.M.; Hijji, Y.M. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT* **2021**, *150*, 111932. [CrossRef]
47. Kim, J.S. Antioxidant activity of various soluble melanoidins isolated from black garlic after different thermal processing steps. *Prev. Nutr. Food Sci.* **2020**, *25*, 301–308. [CrossRef]
48. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*; Iowa State University Press: Ames, IA, USA, 1982.
49. Gomez, K.A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*; John Wiley & Sons: New York, NY, USA, 1984.
50. Berg, G.; Rybakova, D.; Fischer, D.; Cernava, T.; Vergès, M.C.C.; Charles, T.; Schloter, M. Microbiome definition re-visited: Old concepts and new challenges. *Microbiome* **2020**, *8*, 103. [CrossRef]
51. Oukala, N.; Aissat, K.; Pastor, V. Bacterial endophytes: The hidden actors in plant immune responses. *Plants* **2021**, *10*, 1012. [CrossRef]
52. Al-Sayed, H.M.; Hegab, S.A.; Youssef, M.A.; Khalafalla, M.Y.; Almaroai, Y.A.; Ding, Z.; Eissa, M.A. Evaluation of quality and growth of roselle (*Hibiscus sabdariffa* L.) as affected by bio-fertilizers. *J. Plant Nutr.* **2020**, *43*, 1025–1035. [CrossRef]
53. Riddech, N.; Ma, Y.N.; Yodpet, B. Enhancing growth of roselle (*Hibiscus sabdariffa* L.) using stress-tolerant rhizobacteria biofertilizer. *Int. J. Environ. Res.* **2024**, *18*, 26. [CrossRef]
54. Silva, C.F.; Vitorino, L.C.; Mendonça, M.A.C.; Araújo, W.L.; Dourado, M.N.; Albuquerque, L.C.; Souchie, E.L. Screening of plant growth-promoting endophytic bacteria from *Aloe vera*. *S. Afr. J. Bot.* **2020**, *134*, 3–16. [CrossRef]
55. Kukla, M.; Płociniczak, T.; Piotrowska-Seget, Z. Diversity of endophytic bacteria in Lolium perenne and their potential to degrade petroleum hydrocarbons and promote plant growth. *Chemosphere* **2014**, *117*, 40–46. [CrossRef]
56. Etminani, F.; Harighi, B. Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. *Plant Pathol. J.* **2018**, *34*, 208–217. [CrossRef]
57. Shan, W.; Zhou, Y.; Liu, H.; Yu, X. Endophytic actinomycetes from tea plants: Isolation and plant growth-promoting activities. *BioMed Res. Int.* **2018**, *2018*, 1470305. [CrossRef]
58. Borah, A.; Das, R.; Mazumdar, R.; Thakur, D. Culturable endophytic bacteria of Camellia species endowed with plant growth promoting characteristics. *J. Appl. Microbiol.* **2019**, *127*, 825–844. [CrossRef]
59. Kabir, M.H.; Unban, K.; Kodchasee, P.; Govindarajan, R.K.; Lumyong, S.; Suwannarach, N.; Khanongnuch, C. Endophytic bacteria isolated from tea leaves (*Camellia sinensis* var. assamica) enhanced plant-growth-promoting activity. *Agriculture* **2023**, *13*, 533. [CrossRef]
60. Esmaeilian, Y.; Babaeian, M.; Iriti, M. Towards organic farming in roselle (*Hibiscus sabdariffa* L.) cultivation: Feasibility of changing its nutrition management from chemical to bio-organic. *J. Plant Nutr.* **2024**, *47*, 3144–3164. [CrossRef]

61. Alraey, D.A.; Haroun, S.A.; Omar, M.N.; Abd-El Gawad, A.M.; El-Shobaky, A.M.; Mowafy, A.M. Fluctuation of essential oil constituents in Origanum syriacum subsp. sinaicum in response to plant growth promoting bacteria. *J. Essent. Oil-Bear. Plants* **2019**, *22*, 1022–1033. [CrossRef]
62. Ordookhani, K.; Sharafzadeh, S.; Zare, M. Influence of PGPR on growth, essential oil and nutrient uptake of sweet basil. *Adv. Environ. Biol.* **2011**, *5*, 672–677.
63. Mukhtar, T.; Rehman, S.U.; Smith, D.; Sultan, T.; Seleiman, M.F.; Alsadon, A.A.; Saad, M.A. Mitigation of heat stress in Solanum lycopersicum L. by ACC-deaminase producing Bacillus cereus. *Sustainability* **2020**, *12*, 2159. [CrossRef]
64. Shalan, M.N.; Abd-Ellatif, T.A.; Soliman, A.G.; El-Gaawwas, E.O. Effect of chemical and biofertilizer treatments on roselle plants. *Egypt. J. Agric. Res.* **2001**, *79*, 587–606. [CrossRef]
65. Hassan, F.A.S. Response of *Hibiscus sabdariffa* L. plant to some biofertilization treatments. *Ann. Agric. Sci.* **2009**, *54*, 437–446.
66. Vashvaei, R.M.; Ghanbari, A.; Fakheri, B.A. Effect of bio-fertilizers on growth and sepals yield of roselle. *Agroecology* **2017**, *9*, 276–295.
67. Gupta, R.; Pandey, R. Microbial interference ameliorates essential oil yield and diminishes root-knot infestation in sweet basil under field conditions. *Biocontrol Sci. Technol.* **2015**, *25*, 1165–1179. [CrossRef]
68. Ibrahim, E.; Fouad, H.; Zhang, M.; Zhang, Y.; Qiu, W.; Yan, C.; Chen, J. Biosynthesis of silver nanoparticles using endophytic bacteria and their role in inhibition of rice pathogenic bacteria and plant growth promotion. *RSC Adv.* **2019**, *9*, 29293–29302. [CrossRef]