RESEARCH ARTICLE OPEN ACCESS

ASWAN JOURNAL OF AGRICULTURAL AND BIOLOGICAL SCIENCES
Aswan University, Faculty of Agriculture and Natural Resources, Egypt
10.21608/AJABS.2025.404381.1014

Integrating self-endophytic bio-inoculant to enhance Roselle productivity under reduced chemical fertilization in Aswan, Egypt

Zohor A. Ibrahim[1], Wagdi S. Soliman[*2], Osama Konswoa [3], Mohammed T. Abbas [1]

[1]Department of Agricultural Microbiology, Faculty of Agriculture and Natural Resources, Aswan University.
[2] Department of Horticulture, Faculty of Agriculture and Natural Resources, Aswan University, Egypt. Department of Biochemistry, [3] Faculty of Agriculture, Cairo University.

*** Corresponding author**

Wagdi S. Soliman

wagdi79@agr.aswu.edu.eg

10.21608/ajabs.2025.404381.1014

Received: 15/07/2025

Revised: 21/07/2025

Accepted: 30/07/2025

Published: 16/09/2025

Abstract

Roselle (*Hibiscus sabdariffa* L.) is a valuable medicinal and economic crop widely cultivated in Egypt, with Aswan contributing 53% of the total area. On the other hand, sustainable agriculture increasingly relies on plant-microbe interactions, particularly endophytic microorganisms. This study investigated the potential of self-endophytic microbiomes as a bioformulation to reduce dependence on chemical fertilizers while enhancing roselle growth and productivity. A field experiment was conducted using different NPK fertilizer levels combined with a mixed self-endophytic inoculum. Results showed that replacing 25% of NPK with bio-inoculation significantly increased fruit number and shoot biomass by 25% and 15%, respectively. Also, using 75% NPK plus bio-inoculation improved seed weight and dry calyx yield by 35% and 16%, respectively, compared to full NPK. Additionally, this treatment enhanced chlorophyll a and b content by 15% and 23%. The highest carotenoid content was observed with 25% NPK plus bio-inoculant with 21% increase. These findings confirm that endophytic bacteria can colonize roselle tissues without causing harm, improve nutrient uptake, and boost productivity. This eco-friendly approach represents a promising strategy for sustainable roselle cultivation, reducing chemical input while maintaining high yields and supporting environmental health.

Keywords: *Hibiscus sabdariffa*; PGPR; Endophytic; Rhizobacteria; Bioformulation.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is a medicinal plant belonging to the family Malvaceae, commonly known as karkade. It is believed to be native to West Africa and has been widely distributed across various regions worldwide. Roselle is an herbaceous shrub that grows to a height of 1.5–2 meters (Dhar et al., 2015). Although roselle is cultivated in several Egyptian governorates, Aswan is the primary hub for its cultivation, accounting for approximately 53% of Egypt's total cultivated area (CAPMAS, 2023).

Roselle is valued for its stems, leaves, calyxes, and seeds, all of which have industrial, medicinal, and nutritional applications. The calyxes contain organic acids (tartaric, oxalic, malic, ascorbic, and succinic acids), glucose, β-carotene, lycopene, and anthocyanins such as delphinidin and cyanidin (Gaafar et al., 2021). In addition to its numerous therapeutic properties, roselle seeds contain 3.2% sterol content, including ergosterol (Hashem et al., 2017). In addition, roselle fruits and calyxes are rich in essential nutrients including vitamin A, vitamin C, minerals, polysaccharides, pectin, β-carotene, and anthocyanins (Ahmad et al., 2010; Fallahi et al., 2017). The plant is particularly known for its anthocyanin pigments, which play a crucial role in protecting it from biotic and abiotic stresses (Middleton et al., 1993).

In recent years, using chemicals increased to address issues with diseases and pests, susceptibility to abiotic stress, and improve the growth and output of medicinal plants by reducing nutrient depletion (Pérez-Jaramillo et al., 2016). Nitrogen, the most often used agent in horticulture and agriculture, is one of the fundamental components that generate yield and could influence the levels of numerous metabolites (Strzemski et al., 2021). Although nitrogen fertilization has varying effects on the yield of cultivated medicinal plants as well as on the synthesis of significant secondary metabolites, it is important to keep in mind that this practice frequently contribute to soil pollution and may result in excessive chemical residues in products derived from medicinal plants (Liao & Xia, 2024). Numerous qualitative problems have emerged as a result of chemical fertilizer use, and the conventional agricultural approach such as decreased plant secondary metabolite production, heightened vulnerability to pests and diseases, decreased nutrient usage efficiency, and inability to withstand abiotic stressors.

In order to cultivate and harvest medicinal plants in sustainable manner, new eco-friendly fertilizers and intercropping techniques should enhance their quantitative and qualitative while maintaining ecosystem balance and preventing the release of hazardous chemicals into the environment (Machiani et al., 2023). Among the most promising sustainable solutions is the utilization of self-endophytic microbiome, which plays a crucial role in enhancing plant resilience, development, and productivity under stressed environmental conditions.

Several studies highlighted the potential of plant-growth-promoting endophytes in alleviating abiotic stress (Li et al., 2019; Sanayei et al., 2021; Kumar et al., 2022). The bacterial genera *Pseudomonas*, *Bacillus*, *Azospirillum*, and *Rhizobium*, as well as fungal endophytes like *Trichoderma* and *Aspergillus*, have been associated with enhanced heat stress tolerance in crops (Khan et al., 2016). Endophytic microbes, including bacteria and fungi residing within plant tissues, establish mutualistic relationships with roselle. They conferred various benefits through enhanced production of heat-shock proteins and antioxidant enzymes, improving water and nutrient uptake by increasing root surface area and solubilizing essential nutrients, induction of systemic resistance to protect against oxidative stress and temperature-induced damage, and regulation of plant hormonal balance (e.g., auxins, gibberellins, and abscisic acid) to maintain growth under stress (Hiruma et al., 2016).

Therefore, this study aimed to utilize a selected group of potent endophytic microbiomes to enhance growth and yield characteristics of roselle under Aswan conditions.

Materials and methods

A. Roselle (*Hibiscus sabdariffa* L.) plants collection

- **Roselle (*Hibiscus sabdariffa* L.) plants collection**

Samples of healthy and mature roselle plants were randomly collected from different locations at the experimental farm of the Faculty of Agriculture and Natural Resources, Aswan University, Egypt)23°59'52"N 32°51'35"E170). Root samples were collected as a source for the isolation of endophytic bacteria. Samples were placed in sterile, sealed bags and transported to the laboratory in an ice box and subjected to processing within 24 hours.

- **Isolation of endophytic bacteria**

Endophytic bacteria were isolated from root samples according to the method of (Fisher et al., 1992; Zou et al., 2023) with some modifications. Root samples (13.0g) were washed with running tap water to remove soil and dust then dried with filter papers. The root segments were transferred to a laminar airflow hood and cut into small slices or pieces for surface sterilization. Sequentially immersed in 75% (v/v) ethanol for 2 min and then washed in sterilized distilled water two times, immersed in 5% (v/v) sodium hypochlorite (NaClO) for 10 min, and finally washed five times with sterile distilled water. Pieces of sterilized roots, with a surface sterilization, were taken, ranging in size from 3 to 5 cm, and were ground and transferred to 90 ml of a sterilized saline solution potassium chloride (0.85%). One to seven 45 ml bottles of saline were serially diluted. They were then cultured on ten nutrient plates, each containing two different types of roselle agar (RA) and nutrient agar (NA). These plates were incubated at 30°C for 24–72 h to count the bacterial population. Following the selection of various bacterial colonies from the third (10-3) dilution plates, colonies were streaked onto plates, purified at least three times in fresh NA agar, and then moved to slant agar. The purified isolates were kept in 70% glycerol at -20°C.

- **Inocula formulation**

Ten potent isolates recording the highest values in PGPR traits (Table 1) were mixed equally to make formulate a composite inoculum ($5x10^6$) in nutrient broth medium. Each isolate was gently shaken for 150 rpm for 1 hour. Seeds of roselle were soaked overnight in mixed inoculum (250 ml for each isolate) before sowing. A boost inoculation dose (10 ml plant^{-1}) was applied after 41 of planting.

Table 1: The selected potent endophytes isolate recorded the highest values in PGP traits under in vitro conditions.

S. NO	Isolates code	IAA mgl^{-1}	N2 assay mgl^{-1}	Solubilization		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

-; Not detected, *PSI; Phosphate Solubilization Index, and **KSI; Potassium Solubilization Index

B. Plant materials and growth condition

A field study was conducted in 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 aim of study was to evaluate the effect of potent endophyte bacterial isolate mixture (10 isolates) on growth and productivity of roselle plants cultivated in newly reclaimed soil under high-temperature.

Roselle seeds were obtained from the Horticulture Department, Faculty of Agriculture and Natural Resources, Aswan University, Egypt. On June 1st, 2024, seeds were sown in soil, each sub-plot measuring 5×1.20 m, consisting of two rows spaced 30 cm apart. Approximately 30 days after sowing, the plants were thinned to maintain two plants per hill, resulting in 64 plants per sub-plot (32 plants per row across two rows). A drip irrigation system was implemented, and all agricultural practices were carried out according to the recommended procedures throughout the growing season.

The soil was sandy, and its physical and chemical properties were analyzed following the methods described by Jackson (1973) and Black et al. (1982), as detailed in (Table 2).

Table 2: Physical and chemical characteristics of the studied soil

Characteristics	Value
Physical properties (%)	
Silt+Clay	3.9
Sandy	96.10
Textural class	Sandy
Chemical properties	
pH (1:2.5 extract)	7.94
EC (dSm^{-1}) at 25°C	0.53
Soluble anions (meq/L)	
CO_3^{-2}	0.0
HCO_3^-	2.7
Cl-	1.0
SO_4^{-2}	2.0
Soluble cations (meq/L)	
Ca^{++}	1.07
Mg^{++}	1.23
K^+	1.3
Na^+	2.1
Available macronutrients (ppm)	
Available N	192.0
Available P	24.0
Available K	185.0

C. Experimental design

A randomized complete block design (RCBD) in factorial design was used in this study with four replicates. The recommended dose of chemical fertilizer (100% NPK) consisted of ammonium nitrate 33.5% (250 kg per feddan), superphosphate 19.5% (150 kg per feddan) and potassium sulphate 49.5% (75 kg per feddan). The bio-formula consisted of ten isolates as described before. The total treatments were six treatments as follows: 1) control, the recommended dose (100% NPK), 2) 75% NPK plus bio-formula, 3) 50% NPK plus bio-formula, 4) 25% NPK plus bio-formula, 5) bio-formula alone, and 6) fertilizer-free (without chemical and bio-formula).

D. Data recorded

- **Morphological characteristics**

After 116 days (blooming stage), five plants from each treatment were harvested in each replicate to record vegetative measures: plant height (cm), plant diameter (mm), branches number, and fruits number. The fresh weights of the calyxes, seeds, and shoots (g $plant^{-1}$) were measured directly after harvesting, and dry weight were measured after air drying for two weeks.

- **Chemical determination**

a) Photosynthetic pigments

Random samples of fresh leaves from healthy plants were collected by selecting the middle parts of the plants for each treatment during the fruiting stage to evaluate the content of chlorophyll "a", chlorophyll "b", and carotenoids **(Metzner *et al*., 1965)**.

b) Mineral Content

For chemical analysis, 0.2 g of dried plant leaves were subjected to wet digestion using a 1:1 (v/v) mixture of concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) in a heating digester (DK, Velp Scientific Srl, Italy). The resulting extracts were used for further chemical analysis.

- **Nitrogen (%)** was determined using the Micro-Kjeldahl method following the procedure described in (Al Sahaf and Fadel,1989).
- **Phosphorus (%)** was measured using the soft digestion method with ammonium molybdate and ascorbic acid and quantified calorimetrically using a SPECTRO star Nano (BMG LAbTECH GmbH, Germany) (John, 1970).
- **Potassium (%)** was estimated to use a flame photometer, according to the method outlined in (Chapman and Pratt, 1961).
- **Calcium (%)** was analyzed using a flame photometer as described in (Pearson, 1976).

E. Statistical Analysis

Data were statistically analyzed using the "F" test, as described by Snedecor and Cochran (1982). Mean comparisons were performed through the Least Significant Difference (L.S.D.) test following the methodology outlined in Gomez and Gomez (1984). Statistical analysis was performed using the R software (version 2023, R Foundation for Statistical Computing, Vienna, Austria).

Results

A. Vegetative characteristics

- **Plant height**

Data in Table (3) showed that partial replacing chemical fertilizers with biofertilizer did not cause significant changes in plant height. The only treatment that exhibited a noticeable decline was without fertilizers (fertilizer-free). The lowest plant height (133 cm) was recorded in the fertilizer-free treatment, while the highest (158 cm) was observed in the 50% NPK + biofertilizer treatment. This suggests that biofertilization performed comparably to the recommended chemical fertilization regime.

Table 3: Effect of different levels of chemical fertilizers with bio-formula on vegetative characteristics of roselle plants.

Treatments	Plant height (cm)	Plant diameter (mm)	Branches number plant^{-1}	Fruits number plant^{-1}
T1	154.5a	15.81a	3.73bc	14.93bc
T2	156.0a	15.31a	4.07ab	17.93a
T3	158.1a	15.20a	4.27a	15.40b
T4	156.3a	14.63ab	3.87abc	18.60a
T5	157.6a	13.41bc	3.40c	15.33bc
T6:	133.6b	12.89c	3.40c	13.33c
L.S.D. 0.05	6.70	1.23	0.48	2.01

T1: Full dose 100% NPK; T2: 75% NPK+Bio; T3: 50% NPK+Bio; T4: 25% NPK+Bio; T5: Bio only; and T6: Fertilizer-free.

- **Plant diameter**

As shown in Table (3), there were no significant differences in plant diameter among the biofertilization treatments combined with varying levels of chemical fertilizer (25, 50, and 75% NPK) and the 100% chemical fertilization treatment (control). This indicates that the proposed chemical and biological fertilization methods were comparable. The highest stem diameter (15.8 mm) was recorded in the 100% chemical fertilization treatment, while notable reductions were observed in the bio-fertilizer treatment (13.4 mm) and the fertilizer-free treatment (12.9 mm).

- **Number of branches**

Table (3) showed that incorporating biofertilizer significantly increased the number of branches per plant compared to the 100% NPK fertilization treatment. The highest values were recorded in the 50% NPK + biofertilizer (4.3) and 75% NPK + biofertilizer (4.1) treatments. In contrast, the lowest number of branches (3.4) was observed in the treatments with biofertilizer alone and fertilizer-free.

- **Number of fruits**

Data in Table (3) indicate that the number of fruits significantly increased as the percentage of chemical fertilizers decreased. When the recommended NPK dose was applied, the fruit count was 14.9. However, with partial replacement by biofertilizer, the number gradually increased, reaching 18.6 in the 25% NPK+ biofertilizer treatment, showing a significant increase compared to the control. Conversely, fertilizer-free treatment exhibited a notable decrease in fruit count, dropping to 13.3.

- **Shoot fresh and dry weight**

Data in Table (4) showed that partially replacing chemical fertilizers with biofertilizer supported vegetative biomass. The highest fresh weight (286 g plant^{-1}) was recorded in the 25% NPK + biofertilizer treatment, with no significant differences compared to the 100% NPK treatment or the 50% and 75% NPK + biofertilizer treatments. However, using biofertilizer alone (220.5 g plant^{-1}) or no fertilizer (206 g plant^{-1}) resulted in notable declines.

For shoot dry weight, a steady increase was observed with partial replacement of chemical fertilizers by biofertilizer. The highest dry weight (68.4 g plant^{-1}) was recorded in the 25% NPK+biofertilizer treatment, whereas significant reductions were noted in the biofertilizer-only (47.9 g plant^{-1}) and fertilizer-free (15.5 g plant^{-1}) treatments compared to the control (100% NPK).

- **Calyxes fresh weight per plant**

As shown in Table (4), the highest fresh weight of calyxes (53.3 g plant^{-1}) was recorded with the100% NPK dose. Partial replacement of 25–75% NPK with biofertilizer maintained high fresh weight levels, with no significant differences compared to the control. However, significant reductions were observed in the biofertilizer treatment (42 g plant^{-1}) and fertilizer-free treatment (35 g plant^{-1}).

- **Calyxes dry weight per plant and yield per feddan**

According to Table (4), fertilization with 75% NPK + biofertilizer resulted in the highest dry weight of calyxes and yield (8.13 g plant^{-1} and 285 kg feddan^{-1}, respectively), achieving a 16% increase compared to the 100% NPK treatment. However, reducing chemical fertilizers by more than 25% led to significant declines, with the lowest dry weight recorded in the biofertilizer-only (4.9 g plant^{-1} and 172.7 kg feddan^{-1}) and fertilizer-free (4.7 g plant^{-1} and 193.3 kg feddan^{-1}) treatments.

- **Fresh and dry weights of seeds**

Table (4) showed that the lowest fresh seed weight (34.5 g plant^{-1}) was recorded with the application of 100% chemical fertilizers, with minimal variation in the fertilizer-free treatment. Fresh seed weight increased with partial substitution of chemical fertilizers with biofertilizer, reaching its highest value in the 75% NPK + biofertilizer treatment (57.9 g plant^{-1}), followed by 50% NPK + biofertilizer treatment (48.7 g plant^{-1}).

Statistical analysis of the dry seed weight data revealed no significant differences among treatments, whether using the recommended chemical fertilizer dose, partial replacement, or no fertilization. The only exception was a significant increase observed in the 75% NPK + biofertilizer treatment compared to the other treatments.

Table 4: Effect of different levels of chemical fertilizer with bio-formula on fresh and dry weights of shoot, calyxes, and seeds of roselle plants.

Treatments	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Calyxes fresh weight (g plant^{-1})	Calyxes dry weight (g plant^{-1})	Seed fresh weight (g plant^{-1})	Seed dry weight (g plant^{-1})	Yield dry (Kg feddan^{-1})
T1	257.4a	59.4c	53.3a	7.00ab	34.5d	11.20b	245.0ab
T2	283.7a	61.5bc	52.3a	8.13a	57.9a	15.13a	284.7a
T3	283.9a	67.7ab	50.8a	6.60bc	48.7b	11.40b	231.0bc
T4	286.4a	68.4a	52.1a	5.73cd	48.0b	11.47b	200.7cd
T5	220.5b	47.9d	42.0b	4.93d	40.5c	10.13b	172.7d
T6	205.9b	15.5e	35.0c	4.67d	38.3cd	11.13b	163.3d
L.S.D. 0.05	35.6	6.4	4.9	1.25	5.5	1.70	43.8

T1: Full dose 100% NPK; T2: 75% NPK+Bio; T3: 50% NPK+Bio; T4: 25% NPK+Bio; T5: Bio only; and T6: Fertilizer-free.

B. Chemical analysis

- **Photosynthetic pigments**

As shown in Table (5), using biofertilizer in combination with 50% or 75% NPK led to a slight increase in chlorophyll "a", whereas applying biofertilizer only or without fertilizers resulted in a noticeable decrease. Chlorophyll "b" remained unaffected regardless of whether chemical fertilizer was fully or partially replaced with biofertilizers or omitted entirely. Carotenoid content was highest in the 25% NPK + biofertilizer treatment compared to control (100% NPK), while the lowest values were recorded in the biofertilizer-only and fertilizer-free treatments.

Table 5: Effect of different levels of chemical fertilizer with bioformula on photosynthetic pigments in roselle leaves.

Treatments	Chlorophyll "a" (µl ml^{-1})	Chlorophyll "b" (µl ml^{-1})	Carotenoids (µl ml^{-1})
T1	2.55ab	1.29a	0.84bc
T2	2.92a	1.58a	0.39e
T3	2.74ab	1.51a	0.99ab
T4	2.49ab	1.39a	1.02a
T5	2.36b	1.32a	0.81c
T6	2.33b	1.30a	0.55d
L.S.D. 0.05	0.56	0.59	0.16

T1: Full dose 100% NPK; T2: 75% NPK+Bio; T3: 50% NPK+Bio; T4: 25% NPK+Bio; T5: Bio only; and T6: Fertilizer-free.

- **Minerals (N-P-K) content:**

The highest uptake levels of nitrogen (1.56%), phosphorus (0.26%), potassium (1.24%), and calcium (2.95%) were recorded in 100% NPK treatment (Table 6). The mineral content gradually decreased with partial replacement of chemical fertilizers, showing noticeable variations. The lowest values were observed in biofertilizer-only and fertilizer-free treatments.

Discussion

The experimental findings underscored the significant potential of self-endophytic microbiomes as a sustainable bioformulation capable of reducing dependence on chemical fertilizers together with enhancing plant growth, nutrient accessibility, and improving productivity. These results are consistent with **(**Berg et al., 2020**)** who indicated that the production of bioactive metabolites, resistance to biotic and abiotic stressors, nutrient supply, seed germination and growth, and other aspects of plant holobiont functioning were all influenced by the species- and habitat-specific plant microbiota. Oukala et al. (2021) also indicated that the close connection of soil microbiota with the host results in significant changes in the physiology of the plant, and bacterial endophytes are a vital component of the plant microbiome and are known to support plant health through several processes.

The potent endophytic isolates secured in the present study highlighted their ability to express multiple PGPR traits. These self-endophytic microbiomes can establish a robust microbial

Table 6: Effect of different levels of chemical fertilizer with bioformula on some minerals (nitrogen, phosphorus, potassium and calcium % of roselle leaves).

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)
T1	1.67a	0.27a	1.24a	2.95a
T2	1.52ab	0.22ab	1.04b	2.71ab
T3	1.51ab	0.22ab	1.05b	2.55b
T4	1.49ab	0.21b	1.01b	2.49b
T5	1.47b	0.20b	0.93bc	2.11c
T6	1.57b	0.20b	0.84c	1.15d
L.S.D. 0.05	0.09	0.05	0.14	0.33

T1: Full dose 100% NPK; T2: 75% NPK+Bio; T3: 50% NPK+Bio; T4: 25% NPK+Bio; T5: Bio only; and T6: Fertilizer-free consortium that not only enhances the vegetative growth of crops such as roselle (*Hibiscus sabdariffa*) but also improves nutrient acquisition under stress conditions. These results are consistent with previous findings on roselle plants **(**Abo-Baker and Mostafa, 2011; Al-Sayed et al., 2020; Riddech et al., 2024**)**, *Aloe vera* **(**Silva et al., 2020**)**, *Lolium perenne* **(**Kukla et al., 2014**)**, wild pistachio trees **(**Etminani and Harighi, 2018**),** and *Camellia sinensis* **(**Shan et al., 2018; Borah et al., 2019; Kabir et al., 2023**)**.

The integration of biofertilizers derived from these potent endophytes enhanced the bioavailability of essential nutrients. The capacity of the selected isolates to fix atmospheric nitrogen and solubilize phosphorus and potassium plays a crucial role in nutrient cycling. This microbial activity has the potential to reduce the reliance on chemical fertilizers. Experimental data indicated that partial replacing chemical fertilizers with biofertilizers, supported, vegetative biomass and yield. Notably, the treatment comprising 25% NPK supplementation with biofertilizer resulted in shoot fresh weight comparable to that achieved with full chemical fertilization, illustrating that strategic biofertilizer application can maintain plant growth while minimizing chemical inputs. These findings align with Esmaeilian et al. (2024) on roselle plant, Alraey et al. (2019) on *Origanum syriacum* subsp. Sinaicum, and Ordookhani et al. (2011) on *Ocimum basilicum*.

Abiotic stresses, particularly high temperatures of Aswan governorate, present a significant limitation to crop productivity. The resilience of the selected endophytic isolates to elevated temperatures offers a critical advantage. Plants inoculated with these robust microbial partners exhibit enhanced stress tolerance, thereby ensuring stable yields under fluctuating environmental conditions. Additionally, these microbes stimulate the production of plant hormones and secondary metabolites, further improving plant resilience. Moreover, the potential of endophytic microbiomes in 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. (2021) who showed better understanding of endophyte bacteria, to be used for medicinal plants to increase yield and resilience to a range of environmental stressors **(**Mukhtar et al., 2020**)** on *Solanum lycopersicum* L.

The integration of self-endophytic microbiomes into crop management strategies aligns with sustainable agricultural practices by reducing reliance on chemical fertilizers. The experimental results indicated that while complete replacement of chemical fertilizers with biofertilizers may lead to low nutrient content and reduced dry biomass, a balanced approach—moderately reducing chemical fertilizers while supplementing with biofertilizers—can optimize yield. In this study, the 75% NPK + biofertilizer treatment not only maintained essential growth parameters but also led to a 16% increase in calyx dry weight and overall yield compared to the full chemical fertilizer regime. This demonstrates that bioformulations based on endophytes can effectively enhance nutrient uptake and productivity while mitigating the environmental impact associated with excessive chemical inputs. These result agreement with previous studies which indicated increased plant height, number of branches, number of fruits/plants, calyxes fresh and dry weights (Shalan et al., 2001; Hassan, 2009; Vashvaei et al., 2017**)**. Also, Esmaeilian et al. (2024) demonstrated that the stability and sustainability of agricultural production are now seriously threatened using chemical fertilizers in farming systems, particularly in dry areas. Therefore, one of the most important aspects of crop management is identifying alternative nutrition systems based on organic and biological sources **(**Gupta and Pandey, 2015**)**, which indicated that bacterial suspensions increase *Ocimum basilicum* plant seed yield enhanced production of new biomass and decreased severity of root-knot disease. In addition, Ibrahim et al. (2019) revealed that *Coriandrum sativum* plants are home to the endophytic bacterium *Bacillus siamensis*, which allows the plants to grow larger with supporting, shoot length, root length, dry weight.

Conclusion

In conclusion, the use of self-endophytic microbiomes as a bioformulation presents a promising avenue for sustainable agriculture, offering a viable strategy to enhance plant growth, nutrient efficiency, and resilience against abiotic stress with reducing chemical fertilizer dependency.

Author Contributions:

Conceptualization, M. T. A. and W. S. S.; methodology and formal analysis, Z. A., O. K., W. S. S. and M. T. A.; investigation, Z. A., O. K., W. S. S. and M. T. A.; writing—original draft preparation, Z. A. and M. T. A.; writing—review and editing, Z. A., O. K., W. S. S., supervision, O. K., W. S. S. and M. T. A., All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding

Acknowledgments

The authors sincerely acknowledge the generous support of Dr Hanan H. Youssef for running joint and valuable advice in the experiments to accomplish this work. Zohor deeply appreciates the continuous support and encouragement from her family and expresses gratitude to her colleagues. Thanks, are also extended to Mrs. Sawsan EL-Nobi for her excellent technical and lab support. Very much appreciated is the laborious support of the undergraduate students; Nour Ahmed, Essam Alaaeldin, Loai Mostafa, Mostafa Yahya, Mina Zakaria throughout the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

Abo-Baker, A. A., Mostafa, G. G. (2011). Effect of bio-and chemical fertilizers on growth, sepals yield and chemical composition of *Hibiscus sabdariffa* at new reclaimed soil of South Valley area. *Asian Journal of Crop Science, 3*(1), 16-25. DOI: 10.3923/ajcs.2011.16.25

Ahmad, N., Sharma, S., Alam, M. K., Singh, V. N., Shamsi, S. F., Mehta, B. R., Fatma, A. (2010). Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids and Surfaces B: *Biointerfaces, 81*(1), 81-86. https://doi.org/10.1016/j.colsurfb.2010.06.029

Alraey, D. A., Haroun, S. A., Omar, M. N., Abd-ElGawad, A. M., El-Shobaky, A. M., Mowafy, A. M. (2019). Fluctuation of essential oil constituents in *Origanum syriacum* subsp. *sinaicum* in response to plant growth promoting bacteria. *Journal of Essential Oil-Bearing Plants, 22*(4), 1022-1033. https://doi.org/10.1080/0972060X.2019.1661794

Al-Sahaf, H. F., Fadel, H. (1989). Applied Plant Nutrition, Ministry of Higher Education and Scientific Research. *The University of Baghdad. House of Wisdom. Mosul. Iraq, 2*(6), 1-12.

Al-Sayed, H. M., Hegab, S. A., Youssef, M. A., Khalafalla, M. Y., Almaroai, Y. A., Ding, Z., Eissa, M. A. (2020). Evaluation of quality and growth of roselle (*Hibiscus sabdariffa* L.) as affected by bio-fertilizers. *Journal of plant nutrition, 43*(7), 1025-1035.

https://doi.org/10.1080/01904167.2020.1711938

Machiani A. M., Javanmard, A., Ostadi, A., Alizadeh, K. (2023). 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, 12*(7), 1422.

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. Microbiome, 8, 1-22.

Black, C.A., Evans, D.., Ensminger, L. E., . White, J. L., Clark, F. E., Dinauer, R.C. (1982). Methods of Soil Analysis. part 2. Chemical and microbiological properties. 2nd Ed. Soil Sci., Soc. of Am. Inc. Publ., Madison, Wisconsin, U.S.A.

Borah, A., Das, R., Mazumdar, R., Thakur, D. (2019). Culturable endophytic bacteria of Camellia species endowed with plant growth promoting characteristics. *Journal of applied microbiology,* 127(3), 825-844.

CAPMAS (2023): Central Agency for Public Mobilization and Statistics, Egypt.

Chapman, H. D., Pratt, P. F. (1961). Methods of Soil, Plants and Water Analysis. Univ. California, Div. Agric. Sci., 314p.

Dhar, P., Kar, C. S., Ojha, D., Pandey, S. K., Mitra, J. (2015). Chemistry, phytotechnology, pharmacology and nutraceutical functions of kenaf (*Hibiscus cannabinus* L.) and roselle (*Hibiscus sabdariffa* L.) seed oil: An overview. *Industrial Crops and Products, 77*, 323- 332.

Esmaeilian, Y., Babaeian, M., Iriti, M. (2024). Towards organic farming in roselle (*Hibiscus sabdariffa* L.) cultivation-feasibility of changing its nutrition management from chemical to bio-organic. *Journal of Plant Nutrition, 47*(19), 3144-3164.

Etminani, F., Harighi, B. (2018). Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. *The Plant Pathology Journal, 34*(3), 208.

Fallahi, H. R., Ghorbany, M., Aghhavani-Shajari, M., Samadzadeh, A., Asadian, A. H. (2017). Qualitative response of roselle to planting methods, humic acid application, mycorrhizal inoculation and irrigation management. *Journal of Crop Improvement*, 31(2), 192-208.

Fisher, P. J., Petrini, O., Scott, H. L. (1992). The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *New Phytologist, 122*(2), 299-305.

Gomez, K. A., Gomez, A. A. (1984). Statistical procedures for agricultural research. *John wiley & sons.*

Gupta, R., Pandey, R. (2015). Microbial interference ameliorates essential oil yield and diminishes root-knot infestation in sweet basil under field conditions. Biocontrol *Science and Technology, 25*(10), 1165-1179.

Hashem, H. A. E. A., EL-Hadidy, A. E., Ali, E. A. (2017). Impact of some safe agricultural treatments on insect pests, vascular wilt disease management and Roselle (*Hibiscus sabdariffa* L.) productivity under Siwa Oasis conditions. *International Journal of Environ, 6*(4), 139-162.

Hassan, F. A. S. (2009). Response of *Hibiscus sabdariffa* L. plant to some biofertilization treatments. *Annals of Agricultural Science (Cairo), 54* (2) 437-446.

Hiruma, K., Gerlach, N., Sacristán, S., Nakano, R. T., Hacquard, S., Kracher, B., Schulze-Lefert, P. (2016). Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. *Cell, 165*(2), 464-474.

Ibrahim, E., Fouad, H., Zhang, M., Zhang, Y., Qiu, W., Yan, C., Chen, J. (2019). Biosynthesis of silver nanoparticles using endophytic bacteria and their role in inhibition of rice pathogenic bacteria and plant growth promotion. *RSC advances, 9*(50), 29293-29299.

Jackson, M. L. (1973). Soil chemical analysis, pentice hall of India Pvt. Ltd., New Delhi, India, 498, 151-154.

John, M. K. (1970). Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Science*, 109(4), 214-220.

Kabir, M. H., Unban, K., Kodchasee, P., Govindarajan, R. K., Lumyong, S., Suwannarach, N.,Khanongnuch, C. (2023). Endophytic bacteria isolated from tea leaves (*Camellia sinensis* var. *assamica*) enhanced plant-growth-promoting activity. *Agriculture, 13*(3), 533.

Khan, A.L., Waqas, M., Kang, S.M., Hamayun, M., Lee, I.J. (2016). Plant-growth-promoting endophytic fungi: A sustainable approach for enhancing stress tolerance in crop plants. *Journal of Microbial Biotechnology, 29*(2), 123-135.

Kukla, M., Płociniczak, T., Piotrowska-Seget, Z. (2014). Diversity of endophytic bacteria in *Lolium perenne* and their potential to degrade petroleum hydrocarbons and promote plant growth. *Chemosphere, 117*, 40-46.

Kumar, R., Swapnil, P., Meena, M., Selpair, S., Yadav, B. G. (2022). Plant growth-promoting rhizobacteria (PGPR): Approaches to alleviate abiotic stresses for enhancement of growth and development of medicinal plants. *Sustainability, 14*(23), 15514.

Li, F., He, X., Sun, Y., Zhang, X., Tang, X., Li, Y., Yi, Y. (2019). Distinct endophytes are used by diverse plants for adaptation to karst regions. *Scientific Reports, 9*(1), 5246.

Liao, J., & Xia, P. (2024). Continuous cropping obstacles of medicinal plants: Focus on the plant-soil-microbe interaction system in the rhizosphere. Scientia *Horticulturae, 328*, 112927.

Metzner, H., Rau, H., Senger, H. (1965). Studies on synchronization of some pigment-deficient Chlorella mutants. *Planta, 65*, 186-194.

Middleton, E., Kandaswami, C., Harborne, J. (1993). The flavonoids: advances in research since 1986. Chapman & Hall/CRC, New York.

Gaafar, D. E. M., Baka, Z. A. M., Abou-Dobara, M. I., Shehata, H. S., & El-Tapey, H. M. A. (2021). Microbial impact on growth and yield of *Hibiscus sabdariffa* L. and sandy soil fertility. Egyptian *Journal of Soil Science, 61*(2), 259-274.

Mukhtar, T., Rehman, S. U., Smith, D., Sultan, T., Seleiman, M. F., Alsadon, A. A., Saad, M. A. (2020). Mitigation of heat stress in *Solanum lycopersicum* L. by ACC-deaminase and exopolysaccharide producing Bacillus cereus: effects on biochemical profiling. *Sustainability, 12*(6), 2159.

Ordookhani, K., Sharafzadeh, S., Zare, M. (2011). Influence of PGPR on growth, essential oil and nutrients uptake of sweet basil. *Advances in Environmental Biology, 5*(4), 672-677.

Oukala, N., Aissat, K., Pastor, V. (2021). Bacterial endophytes: The hidden actor in plant immune responses against biotic stress. *Plants, 10*(5), 1012.

Pearson, D. (1976). The chemical analysis of foods. (7th edn) Churchill Livingstone. Edinburgh London, UK.

Pérez-Jaramillo, J. E., Mendes, R., Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology, 90*, 635-644.

Riddech, N., Ma, Y. N., & Yodpet, B. (2024). Enhancing growth of roselle plants (*Hibiscus sabdariffa* L.) using a salt-and drought-tolerant rhizobacteria-soybean biofertilizer. International *Journal of Environmental Research, 18*(2), 26.

Sanayei, S., Barmaki, M., Ebadi, A., Torabi-Giglou, M. (2021). Amelioration of water deficiency stress in roselle (*Hibiscus sabdariffa*) by arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria. Notulae Botanicae Horti Agrobotanici *Cluj-Napoca, 49*(2), 11987-11987.

Shalan, M. N., Abd-Ellatif, T. A., Soliman, A. G., El-Gaawwas, E. O. (2001). Effect of some chemical and biofertilizer treatments on roselle plants. Egyptian *Journal of Agricultural Research, 79*(2), 587-606.

Shan, W., Zhou, Y., Liu, H., Yu, X. (2018). Endophytic actinomycetes from tea plants (*Camellia sinensis*): isolation, abundance, antimicrobial, and plant-growth-promoting activities. *BioMed research international*,1, 1470305.

Silva, C. F. D., Vitorino, L. C., Mendonça, M. A. C., Araújo, W. L. D., Dourado, M. N., Albuquerque, L. C. D., Souchie, E. L. (2020). Screening of plant growth-promoting endophytic bacteria from the roots of the medicinal plant Aloe vera. *South African Journal of Botany, 134*, 3-16.

Snedecor, G.W., Cochran, W. G. (1982). Statistical Methods. Iowa, U.S.A: The Iowa State Univ. Press.

Strzemski, M., Dzida, K., Dresler, S., Sowa, I., Kurzepa, J., Szymczak, G., Wójciak, M. (2021). Nitrogen fertilisation decreases the yield of bioactive compounds in *Carlina acaulis* L. grown in the field. *Industrial Crops and Products, 170*, 113698. https://doi.org/10.1016/j.indcrop.2021.113698

Vashvaei, R. M., Ghanbari, A., Fakheri, B. A. (2017). Effect of bio-fertilizers in combination with different rates of chemical fertilizers on the growth characters and sepals yield of roselle (*Hibiscus sabdariffa* L.). *Agroecology, 9*(2), 276-295

Wu, W., Chen, W., Liu, S., Wu, J., Zhu, Y., Qin, L., Zhu, B. (2021). Beneficial relationships between endophytic bacteria and medicinal plants. *Frontiers in plant science, 12*, 646146.

Zou, L., Wang, Q., Li, M., Wang, S., Ye, K., Dai, W., Huang, J. (2023). Culturable bacterial endophytes of *Aconitum carmichaelii* Debx. were diverse in phylogeny, plant growth promotion, and antifungal potential. *Frontiers in Microbiology, 14*, 1192932. doi: 10.3389/fmicb.2023.1192932