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To What Extent Can Biofertilizers Replace Chemical Fertilizers for Valencia Orange (*Citrus sinensis* L. Osbeck) Trees Growing in Sandy Soil Conditions?

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EXCESSIVE consumption of chemical fertilizers in citrus orchards and other fruit crops has caused many negative effects on the surrounding ecosystem, soil composition, fruit quality, and sustainability. In this investigation, two sources of N, K biofertilizers were added as a partial alternative to mineral nitrogen at levels of 100, 75, and 50% of its recommended dose, and the effect of this on Valencia orange trees' vegetative growth, productivity, and fruit quality was studied. The results indicated that using Bio N with or without Bio K fertilizer, with 75% or 50% of the recommended N fertilizer, enhanced most of the vegetative, yield, and fruit parameters. Moreover, the application of biofertilizers enhanced the mineral contents in leaves. The purpose of this study is to increase Valencia orange trees' capacity for growth in sandy soil without using an excessive amount of N mineral fertilizer.

Keywords: Citrus, Biofertilizers, Sandy soil, Nitrogen-fixing bacteria, Potassium-dissolving bacteria**.**

Introduction

Citrus is Egypt's most important economic fruit crop, according to the Ministry of Agriculture and Land Reclamation's Annual Reports of the Economic Affairs Sector (2023). That's owing to its considerable worth for local markets and export demands. It occupies 529405 feddan in total area, with 482271 feddan fruiting, producing 5142829 tons of fruit annually. Within this area, 144419 feddan are planted with Valencia orange trees, representing 29.9% of the entire citrus and 41.2% of the total cultivated area of orange varieties, coming second after the Washington navel orange (47.06%). This cultivar is considered the most important sweet orange variety in Egypt because of its medium-sized and high-quality fruits, along with its ideal maturation season (February - October), making it particularly suitable for industry and exportation (**El-Khwaga and Maklad, 2013; El-Badawy, 2017; El-Khwaga et al., 2021; Abbas and Abdulhakim, 2024).** Citrus needs sixteen vital elements for ideal growth, fruit quality and yield, and (**Ouaggio et al., 2002**). The primary component of mineral fertilizer for citrus trees is nitrogen. It has a more significant impact on plant productivity and growth. **(Ennab, 2016**). Potassium is essential for basic physiological processes like sugar translocation, cell division, protein synthesis, and growth. It enhances flavor, size, and color, and is vital for fruit development. (**Srivastava, 2012; Shaaban and Abdel-Ati, 2025**).

Generally, there are negative consequences to using chemical fertilizers excessively on crop growth, productivity, and the surrounding ecosystem. This results in soil degradation and environmental pollution, leading to the search for safer, eco-friendly techniques such as biofertilizers and organic fertilization (**Alalaf et al., 2023; Khairi and Joody, 2025).** Finding ways to improve the sustainability of agriculture is crucial for providing enough food while protecting the environment for future generations **(Wang et al., 2022; Hu et al., 2023; Fatima and Al- hadethi, 2024).** Egypt has faced a serious challenge for many years due to the overuse of mineral fertilizers, with fertilizer use per cultivated area being ten times higher than the global average for all nutrients **(FAO, 1994**). This has negatively affected food safety, human health, and soil fertility. In this context, nitrogen fertilizer efficiency drops to 50%, with most nutrients lost through volatilization, leaching, and denitrification. Additionally, potassium and phosphorus remain insoluble in the soil, lowering their accessibility to just 10% of the soil content **(Kucey et al., 1989; Miller et al., 1990; Alalaf et al., 2023; Cano-Castro et al., 2024)**. Biofertilizers, like nitrogen-fixing bacteria, phosphorus-solubilizing microbes, and potassium-dissolving bacteria, offer a partial, eco-friendly alternative for plant nutrition and pest and disease control. They are essential for improving citrus cultivation and productivity; many studies have confirmed their beneficial effects on vegetative growth, tree yield, healthy fruit production, and enhance tolerance of plant to biotic and abiotic stresses. Moreover, they contribute to sustainable, healthy soil, ecosystem stability, and lower production costs and environmental contamination **(El- Khawaga and Maklad, 2013; El- Badawy, 2017; Fatma and Ahmed, 2020)** for Valencia orange, **(Abd El- Migeed et al., 2007; Eman et al., 2008; Akalaa et al., 2022; Pérez-**

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Piqueres et al.,.2025; Semalulu et al., 2025) for Washington navel orange, **(Fathallah et al., 2021)** for Balady mandarin, and **(Ennab, 2016**) for Eureka lemon. All studies agree that applying biofertilizers effectively increases citrus yield and enhances the fruit's physical and chemical properties by boosting soil organic matter, fertility, and biological activity.

This investigation aims to assess the impact of combining various levels of mineral nitrogen fertilizer with N and K biofertilizer sources on leaf mineral content, vegetative growth, and fruit quality parameters of Valencia orange trees growing in sandy soil conditions. It also seeks to estimate the ability to reduce the recommended N chemical dose in the presence of biofertilizer.

2. Materials and Methods

2.1. Experimental site and soil characteristics

This experiment was conducted at a private orchard in the El Khatatb–ah district, El Sadat City, Menofia Governorate, Egypt (30°21'39.5"N 30°49'11.5"E), over two consecutive seasons (2022 and 2023). Thirteen years old Valencia orange *(Citrus sinensis*, L. Osbeck) trees, budded on Volkamer lemon rootstock *(Citrus volkameriana*, L.) and planted 4 x 4 meters apart (625 tree/ha) under a drip irrigation system, were included in this investigation. The soil in the orchard is sandy (Table 1). The standard cultural practices common in this region were applied.

Table 1. Soil physical and chemical characteristics of the experimental site.

Particle size distribution (%)		**- CEC Cmol/kg (7.52) - Saturation, % (19)**	**Available**		**Soluble cation (mmol/L)**				**Soluble anion(mmol/L)**			
Sand	92.63											
Silt	6.8	**EC (dS/m) (1.91)**	**N (ppm)**	**K (ppm)**								
clay	2.01											
Texture of soil		**pH**			**Ca^{++}**	**Mg^{++}**	**Na^{+}**	**K^{+}**	**CO_3**	**HCO_3**	**Cl^{-}**	**SO_4**
(Sandy)		**(7.86)**	15	57	13.08	6.72	9.26	0.58	-	3.88	8.25	15.22

2.2. Experimental treatments

Seventy-two healthy trees were selected for this study based on their comparable vigor and productivity. The selected trees received the recommended horticulture practices except nitrogen fertilization. They were organized in a completely randomized block design with 8 treatments, with tree replicates, three trees per each (Table 2).

Table 2. Experimental scheme description.

Experimental scheme number	Description of the experimental scheme
T_1	Control (100% mineral N)
T_2	(100% min. N + Bio N + Bio K)
T_3	(100% min. N + Bio N)
T_4	(100% min. N +Bio K)
T_5	(75% min. N + Bio N +Bio K)
T_6	(75% min. N + Bio N)
T_7	(50% min. N + Bio N +Bio K)
T_8	(50% min. N+ Bio N)

Mineral nitrogen, phosphorus, and potassium fertilizers at the recommended dose (100%) were applied at rates of 1 kg N, 0.25 kg P_2O_5, and 0.5 kg K_2O /tree/year, respectively.

2.3. Microbial strains

Azotobacter chroococcum (Az14), a nonsymbiotic nitrogen-fixing bacterium, and *Bacillus circulans* (B4), as potassium-dissolving bacteria, were obtained from the Bacteriology Laboratory, Sakha Agricultural Research Station. Pure cultures were routinely kept on Jensen's medium **(1951)** and nutrient agar medium **(Atlas, 1997)**.

Inoculum preparation: Sterilized peat moss (as carrier materials) in sealed bags was injected aseptically with a suitable broth culture that produces 10^7-10^8 CFU ml^{-1}. From every inoculum, 3 kg fed^{-1} was blended with appropriate sandy soil and then spread in crescentic trenches around every tree once during the first week of April of each season. The mineral fertilizers of P, K, and other microelements were applied to all treatments following the Ministry of Agriculture's recommendations based on soil type, irrigation system, and age of the selected trees.

2.4. Vegetative growth parameters measurements

Trunk circumference (cm) was measured in mid-October each season, 30 cm above the grafting area. Thirty mature leaves from non-bearing shoots spread around the tree was sampled to estimate the average leaf area (cm^2) with a portable leaf area meter (Model Cl-202). Chlorophyll content (SPAD) was measured using a CCM-200 plus Opti-Sciences chlorophyll meter. Additionally, leaf fresh and dry weights, N, P, K, Zn, Mn, and Fe contents were estimated. Four branches distributed around each tree were labeled at the end of February of both seasons to determine the increase in shoot length during each growth season.

2.5. Yield and fruit quality parameters

At harvest time, the number of fruits per tree was recorded, and the average fruit weight was used to estimate the average yield /tree (kg) and /feddan (ton). From each tree ten fruits were randomly selected (totaling 90 fruits/treatment) to assess their chemical and physical parameters. Fruit physical properties included fruit diameter (cm) and peel thickness (mm) that were measured with a Vernier caliper. Fruit size (cm^3) and juice volume (cm^3) were determined using a measuring cylinder. Fruit chemical properties measured included total soluble solids (TSS) using a hand refractometer. Titratable acidity percentage (as citric acid), and vitamin C (ascorbic acid) were determined according to **A.O.A.C. (2005).**

2.6. Leaf mineral content

Leaf samples that have previously dried are also used to determine mineral concentration. Dried leaves were digested with hydrogen peroxide and sulfuric acid as described by **Evenhuis and Dewaard (1980)**. Nitrogen, phosphorus, and potassium percentages were measured in the digested solution, following **Mertens (2005)**. Iron, manganese, and zinc concentrations as part per million, were assessed by using electrothermal atomic absorption spectrometer, Perkin Elmer (Model 5100), as described by **Kumpulainen et al. (1983**).

2.7. Statistical analyses

The obtained data were analyzed using the Co-Stat program in a randomized complete block design (RCBD) according to **Snedecor and Cochran (1980)**. Means comparison were performed using Duncan's multiple range test at $P \leq 0.05$ **(Duncan 1955**).

3. Results

3.1. Tree circumference, shoot length, and chlorophyll content (SPAD)

Data in Table 3 shows a positive effect of biofertilizers on enhancing the studied growth parameters during the two seasons under investigation. Tree circumferences were not negatively affected by reducing the N mineral fertilizer level to 75% and 50% of the recommended dose, especially in the presence of N biofertilizer with or without K biofertilizer sources, except for T4 treatment in the 1st season, which had the lowest value. T6 achieved the highest tree circumference in both seasons, followed by T8. Also, the net increment in shoot length during the growth season of both studied years was not affected badly by decreasing mineral N level compared with the control in the presence of biofertilizers, except at the 50% level, where the shoot length recorded the lowest values even in the presence of the Bio N resource. Chlorophyll content was also enhanced by combining biofertilizer with mineral N, as shown in the table. T3 and T4 recorded the highest values during the first and second seasons, respectively, while the control treatment gave the lowest values (Fig. 1).

Table 3. Tree circumference and shoot length of Valencia orange trees as influenced by nitrogen and potassium biofertilizers combined with different levels of nitrogen mineral fertilizer.

Treatments	Tree circumference (cm)		Shoot length (cm)	
	2022	2023	2022	2023
T1	45.3	47.3	92.9 b	92.16 bc
T2	46.3	49.3	92.5 b	93.4 ab
T3	47.7	49.3	95.3 a	95.3 a
T4	44.0	48.6	91.2 b	89.3 cd
T5	48.3	48.3	91.5 b	91.4 bcd
T6	52.7	54.7	92.0 b	91.6 bcd
T7	50.0	48.7	91.3 b	93.3 ab
T8	50.7	49.7	89.0 c	89.2 d
F. test	NS	NS	***	**

In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N),T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N + Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N + Bio K), T8: (50% min. N + Bio N)

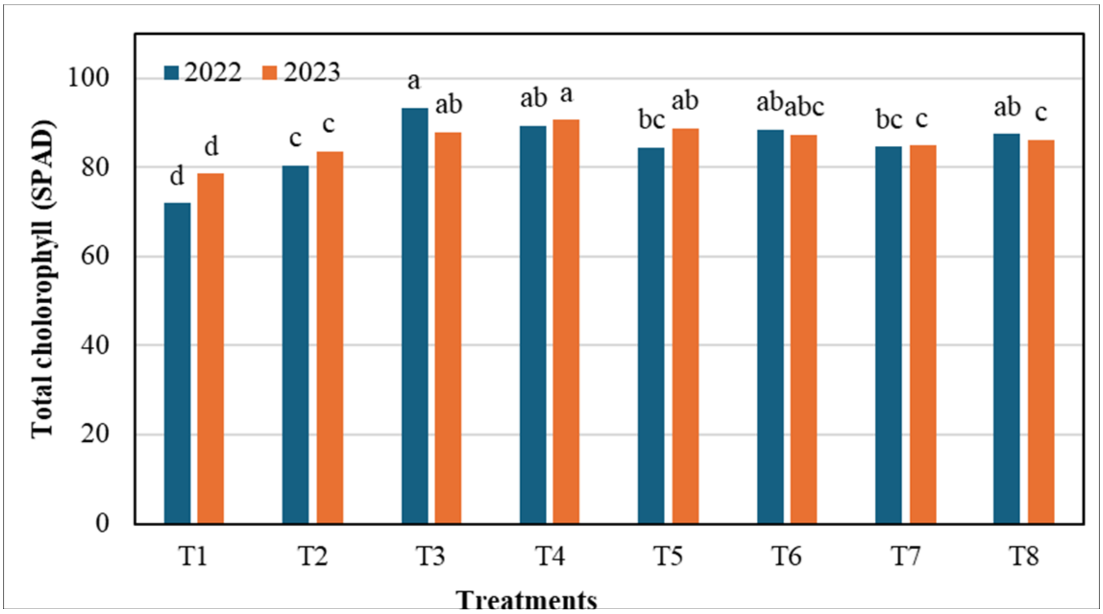

Fig. 1. Total chlorophyll (SPAD) as influenced by nitrogen and potassium biofertilizers in combination with various levels of nitrogen mineral fertilizer.

T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N), T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N + Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N + Bio K), T8: (50% min. N + Bio N**)**

3.2. Leaf area, fresh and dry weights

Data presented in Table 4 show that T8 (50% min. N + Bio N) exhibited the highest average leaf area in both seasons, whereas the control treatment showed the lowest leaf area. In both seasons, leaf fresh and dry weights

Table 4. Leaf area (cm²), leaf fresh and leaf dry weights of Valencia orange trees as affected by nitrogen and potassium biofertilizers in combination with various levels of nitrogen mineral fertilizer.

Treatments	Leaf area (cm²)		Leaf fresh weight (g)		Leaf dry weight (g)	
	2022	2023	2022	2023	2022	2023
T1	18.8 c	19.3 d	7.24 bc	9.35 c	3.63 bc	3.86 c
T2	20.2 b	22.7 a	8.11 b	11.16 b	4.32 b	4.52 ab
T3	19.9 b	22.9 a	6.49 c	10.25 b	3.69 bc	4.38 b
T4	20.3 b	22.5 a	7.75 bc	11.16 b	3.53 c	4.64 ab
T5	20.5 b	21.4 bc	8.08 b	11.06 b	4.03 bc	4.55 ab
T6	19.9 b	22.1 ab	6.58 c	11.24 b	4.29 bc	4.41 b
T7	20.8 b	20.8 c	11.60 a	11.36 b	5.35 a	4.97 a
T8	22.6 a	22.9 a	11.84 a	12.69 a	4.39 b	4.94 a
F. test	***	***	***	**	**	*

Means within each column followed by the same letter are not significantly different at $P \leq 0.05$
T1: Control (100% mineral N), T2: (100%% min. N + Bio N + Bio K), T3: (100% min. N + Bio N),T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N +Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N +Bio K), T8: (50% min. N + Bio N)

3.3. Yield parameters

Results in Table 5 show that adding or partially replacing N mineral fertilizer with bio-N, with or without bio-K, successfully maintained yield parameters at satisfactory to superior levels compared with the control. In the first season, T6 followed by T3 and T7; in the second season, T2, T8, and T7 had the highest fruit numbers per tree. Regarding fruit weight, T8 followed by T2 showed the highest weight in the first season, whereas T2 and T3 recorded the highest values, in the second one. T6 and T8 had the highest yield per tree and per feddan (Fig. 2) in the first season, while T2, followed by T6, performed the highest in yield in the second season. The control treatment produced the least yield in both seasons.

Table 5. Fruit number per tree, fruit weight, and yield per tree as affected by N and K biofertilizers in combination with various levels of N mineral fertilizer .

Treatments	No. of fruits/Tree		Fruit weight (g)		Yield (Kg/tree)	
	2022	2023	2022	2023	2022	2023
T1	210.3 c	210.3 c	190.4 e	215.3 c	40.05 d	45.2 e
T2	245.7 b	272.7 a	224.5 b	247.4 a	55.8 bc	67.38 a
T3	269.3 ab	248.0 b	216.8 bc	246.8 a	58.36 ab	61.2 bcd
T4	258.0 b	212.0 c	201.1de	235.3 ab	51.85 c	49.93 e
T5	260.0 ab	258.0 ab	209.3 cd	219.7 c	54.38 bc	56.68 d
T6	282.7 a	265.0 ab	221.1 bc	249.6 c	62.49 a	66.13 ab
T7	269.3 ab	270.3 a	216.6 bc	218.6 c	58.29 ab	59.08 cd
T8	253.0 b	272.3 a	246.5 a	230.2 bc	62.27 a	62.6 abc
F. test	***	***	***	***	***	***

In each column, means followed by the same letter are not significantly different at $P \leq 0.05$
T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K),T3: (100% min. N + Bio N), T4: (100% min. N + Bio K),T5: (75 % min. N + Bio N +Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N +Bio K), T8: (50% min. N + Bio N)

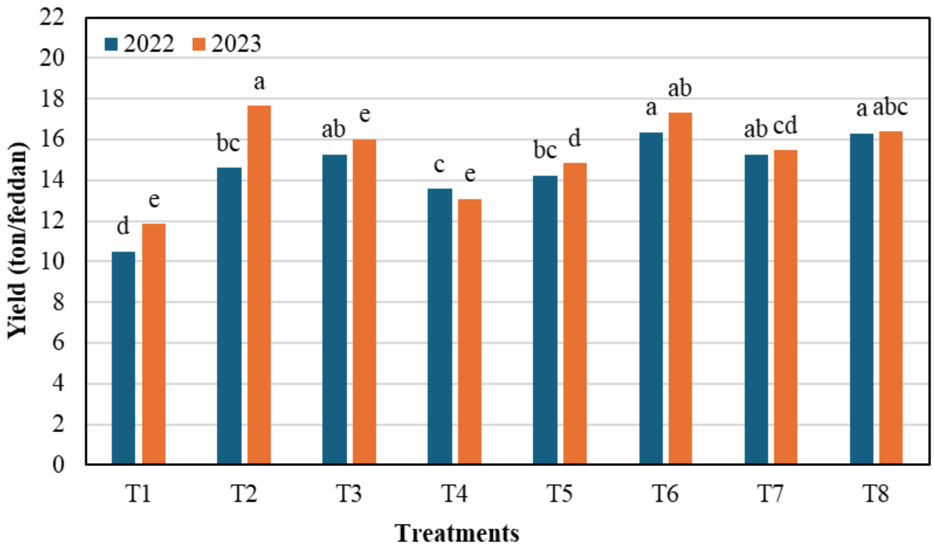

Fig. 2. Yield (ton/feddan) as influenced by nitrogen and potassium biofertilizers in combination with various levels of N mineral fertilizer.

T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N), T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N +Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N +Bio K), T8: (50% min. N + Bio N)

3.4. Fruit physical and chemical properties

Data in Table 6 shows the effect of applied treatments on fruit physical properties indicate a positive impact of adding biofertilizers alongside mineral fertilization sources. The control treatment, exhibited the lowest fruit physical characteristics including fruit diameter, fruit size, peel thickness, and fruit juice volume in both seasons compared to the other treatments. In the first season, T8 produced the highest fruit diameters and did not differ significantly with T7, T5, and T2 treatments, while in the second season T2, T4, T6, and T8 recorded the highest significant fruit diameters. Fruit size increased considerably across all treatments compared with the control in the two seasons. T7 and T8 gave the largest fruit sizes in the first season, while T3 and T4 showed the highest measurements for this parameter, followed by T8 and T4, with no significant differences among them. Regarding peel thickness, T5 had the maximum peel thickness in the two seasons, without significant difference with other treatments except the control and T2 in the first season. Fruit juice volume increased significantly with the use of biofertilizers, especially when combining Bio N with Bio K, as seen in T2 and T5 during the 1st season, and T4 and T2 during the 2nd one.

Table 6. Fruit diameters, fruit size, peel thickness, and juice volume/fruit as affected by N and K Biofertilizers combined with various levels of N mineral fertilizer.

Treatments	Fruit diameter (cm)		Fruit size (cm^3)		Peel thickness (mm)		Juice volume cm^3/fruit	
	2022	2023	2022	2023	2022	2023	2022	2023
T1	6.4 e	7.02 b	129.2 d	169.2 d	2.8 c	2.81 d	74.7 b	75.3 d
T2	6.96 abcd	7.3 a	178.3 b	215.8 a	3.04 b	3.1 bc	89 a	91.7 ab
T3	6.7 cd	7.2 a	152.5 c	218.3 a	3.13 ab	3.18 b	88.7 a	76.7 d
T4	6.7de	7.3 a	156.7 c	206.7 ab	3.2 ab	3.19 b	81.7 ab	96.3 a
T5	7.01 abc	7.01 b	170.8 b	175.0 cd	3.33 a	3.36 a	88.7 a	86.3 bc
T6	6.9 bcd	7.3 a	182.5 b	186.7 bcd	3.21 ab	3.15 bc	84.7 a	86.7 bc
T7	7.14 ab	7.1b	200.8 a	169.2 d	3.13 ab	3.07 c	85.3 a	82.3 cd
T8	7.23 a	7.3 a	196.7 a	207.5 ab	3.19 ab	3.07 c	80.0ab	82.3 cd
F.test	**	***	***	**	**	***	*	***

Means within each column followed by the same letter are not significantly different at $P \leq 0.05$
T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N), T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N + Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N + Bio K) , T8: (50% min. N + Bio N)

Table 7 show the influence of fertilization treatments on the chemical fruit quality parameters. T8 treatment succeeded in keeping TSS level at the same level of the control during both seasons, meanwhile the same treatment (T8) exhibited the lowest acidity values along with T7 in the first season, and T5 and T7 in the second one. T6 had the highest Vitamin C. level followed by T2, T1 and T4, respectively in the 1st season, whereas T4 showed the highest content, followed by T6, in the 2nd season.

Table 7. Total soluble solids, acidity percentage and Vitamin C content as influenced by nitrogen and potassium biofertilizers combined with varying levels of N mineral fertilizer.

Treatments	TSS		Acidity (%)		Vit C (mg/100ml juice)	
	2022	2023	2022	2023	2022	2023
T1	12.4 a	12.1 a	1.85 a	1.44 a	35.95 ab	38.03 e
T2	12.4 a	11.1 bc	1.62 a	1.46 a	37.26 ab	42.69 bcd
T3	11.4 b	10.3 c	1.49 ab	1.16 bc	31.23 b	42.11 cd
T4	11.3 b	11.2 b	1.04 bc	1.14 bc	35.18ab	47.68 a
T5	11.2 b	11.4 ab	1.73 a	0.93 c	29.41 b	43.01 bc
T6	11.3 b	10.9 bc	1.4 abc	1.37 ab	40.29 a	45.73 ab
T7	11.4 b	10.8 bc	0.89 c	0.94 c	28.37 b	38.03 e
T8	12.4 a	12.2 a	0.89 c	1.02 c	29.23 b	39.2 de
F. test	*	**	**	***	*	***

Means within each column followed by the same letter are not significantly different at $P \leq 0.05$
T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N), T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N +Bio K),T6: (75 % min. N + Bio N), T7 (50 % min. N + Bio N +Bio K), T8: (50% min. N + Bio N)

3.5. Leaf mineral constituents

Data in Tables 8 and 9 show that T2 produced the highest nitrogen, phosphate, and potassium contents in both studied seasons, followed by T3. It's clear also that decreasing the mineral N fertilizer level to 75% and 50% of the recommended dose caused a significant deficiency in those macro elements in leaves, as T5, T7, and T8 showed, compared with the control treatments. It's also obvious that adding both types of studied biofertilizers to the recommended dose was perfect for enhancing nitrogen, phosphate, and potassium contents in Valencia orange leaves.

Table 8. Leaf nitrogen, phosphorus and potassium contents as affected by N and K biofertilizers combined with various levels of N mineral fertilizer.

Treatments	N%		P%		K%	
	2022	2023	2022	2023	2022	2023
T1	2.72 d	2.65 c	0.239 d	0.239 e	2.24 d	2.18 e
T2	3.12 a	3.05 a	0.281 a	0.28 a	2.88 a	2.81 a
T3	2.92 b	3.04 a	0.273 b	0.273 b	2.59 b	2.70 b
T4	2.58 e	2.71bc	0.225 e	0.251 d	2.12 e	2.70 b
T5	2.37 f	2.31 d	0.181 g	0.202 f	1.81 f	1.76 f
T6	2.84 c	2.77 b	0.252 c	0.258 c	2.34 c	2.28 d
T7	2.38 f	2.18 e	0.191 f	0.190 g	1.87 f	1.84 f
T8	2.37 f	2.66 c	0.181 g	0.236 e	1.74 g	2.46 c
F. test	***	***	***	***	***	***

Means within each column followed by the same letter are not significantly different at $P \leq 0.05$
T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N), T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N +Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N +Bio K), T8: (50% min. N + Bio N)

However, the studied microelements were affected separately, as T2 had the highest Fe content in the two seasons, Mn content in the 2nd season, and Zn in the 1st season. T5 has the highest Mn and Zn contents in the first and second seasons, respectively, while T7 has the highest Mn content in the 1st season. Otherwise, T8 and T7 have the lowest Fe and Zn values in the two seasons and Mn content in the second season.

Table 9. Iron, manganese, and zinc leaf content (ppm) as affected by N and K biofertilizers combined with various levels of N mineral fertilizer.

Treatments	Fe (ppm)		Mn (ppm)		Zn (ppm)	
	2022	2023	2022	2023	2022	2023
T1	147.18 c	149.23 c	32.06 e	39.10 c	39.33 c	32.54 d
T2	152.81 a	151.59 a	34.37 c	41.11 a	40.46 a	34.40 b
T3	151.90 ab	150.56 bc	33.62 d	40.8 ab	40.18 ab	34.16 b
T4	147.04 c	150.11 bc	31.48 f	39.49 c	38.07 d	33.18 c
T5	143.53 de	152.52 ab	30.73 g	41.09 a	37.60 d	35.10 a
T6	150.90 b	149.65 c	33.52 d	40.40 b	39.75 bc	33.37 c
T7	144.54 d	146.01 d	38.09 a	37.9 d	31.54 e	31.52 e
T8	143.07e	144.97 d	37.03 b	37.47 e	30.06f	31.18 e
F. test	***	***	***	***	***	***

Means within each column followed by the same letter are not significantly different at $P \leq 0.05$
T1: Control (100% mineral N), T2: (100% min. N + Bio N + Bio K), T3: (100% min. N + Bio N),T4: (100% min. N + Bio K), T5: (75 % min. N + Bio N +Bio K), T6: (75 % min. N + Bio N), T7: (50 % min. N + Bio N +Bio K), T8: (50% min. N + Bio N)

4. Discussion

The world population is growing and is expected to surpass ten billion by 2050, making food security a major concern. Therefore, enhancing crop productivity under shifting climate conditions is essential for maintaining global food security. Crop yields have traditionally been improved using methods like chemical fertilizers, but these pose several environmental risks. Consequently, many scientists are turning to alternative and safer fertilizers, such as biofertilizers, which are quickly gaining popularity worldwide as a vital part of agricultural practices. Biofertilizers are living microorganisms that promote plant growth, are eco-friendly, and cost-effective for increasing crop production by directly or indirectly supporting plant growth (**Shahwar et al. 2023).**

In this study, vegetative growth traits of Valencia orange trees showed positive responses to biofertilizer applications containing *Azotobacter chroococcum* alone or combined with *Bacillus circulans*. The treatment of (75% min. nitrogen + Bio N) (T6) increased tree circumference by 16.3% and 15.6% in the two seasons, respectively. Furthermore, (100% min. nitrogen + Bio N) (T3) increased chlorophyll content by 29.9%, while T8 boosted leaf area by 20.2% in the first season. Overall, applying *A. chroococcum* alone or with *B. circulans* improved most vegetative traits of Valencia orange trees. The growth increases observed in treatments T3, T4, T6, T7, and T8 are likely due to the biofertilizers used alone or in combination, especially when paired with nutrient-rich mineral nitrogen fertilizers. These improvements may also result from enhanced nutrient use efficiency and soil cation exchange capacity, which promote root development and nutrient uptake (as shown in Tables 1, 8, and 9). These factors facilitate faster cell division and elongation, leading to larger tree size and increased growth **(Ennab, 2016; Abobatta, 2020)**. Findings align with numerous studies, such as those by **El-Deeb et al. (2013)**, **El-Khwaga and Maklad (2013), Hoda et al. (2013), El-Badawy (2017), Fatma and Ahmed (2020)** on Valencia orange, **Eman et al. (2008)**, **Akalaa et al. (2022)** on Washington Navel orange, **Fathallah et al. (2021)** on Balady mandarin, and **Ennab (2016)** on Eureka lemon. These studies demonstrate the beneficial effects of biofertilizers in promoting vegetative growth by enhancing soil fertility, nutrient uptake, and microbial activities such as nitrogen fixation, potassium solubilization, and the secretion of essential soil elements, thereby supporting improved tree growth and crop yield. In the rhizosphere, plants grow most rapidly when beneficial microorganisms, known as plant growth-promoting rhizobacteria (PGPR), like *A. chroococcum* and *B. circulans*, are abundant **(Saleemi et al., 2017)**. Biofertilizers directly enhance plant growth through providing available nutritional elements (nitrogen, phosphorus, potassium, etc.) and improving soil fertility, or by stimulating phytohormone production in plant tissues through nitrogen fixation, potassium dissolution, and nutrient excretion **(El-Badawy, 2017)**. These effects positively influence growth and productivity **(Kumer et al., 2011; Ennab, 2016; Abobatta, 2020; Abobatta and Azazy, 2020; Alalaf et al., 2023; Fatima and Al-Hadethi, 2024; Semalulu et al., 2025)**. When combined with mineral fertilizers, biofertilizers significantly enhance vegetative growth traits, likely due to increased nutrient uptake as shown in Tables 8 and 9, which improve all growth parameters **(Bayoumi, 2005)**. The growth stimulation from applying *A. chroococcum* and/or *B. circulans* with mineral fertilizers may also result from their synergistic effects, increasing vitamins, amino acids, macro- and micro-nutrients, and phytohormones, thus boosting tree growth. Additionally, biofertilizers increase levels of amino acids, vitamins, hormones, and nutrients, which may enhance chlorophyll content and promote higher growth rates in photosynthesis. Regarding the positive effects of biofertilizers on fruit quality and yield, in comparison with the control, T6 and T8 increased the number of fruits/tree by 34.4% and 29.7% in both seasons, respectively. Also, yield/tree and yield/feddan recorded 55.5% and 38.5% increases over the control in both seasons, respectively. They produced higher yields from biofertilizer application than from mineral fertilizers, likely due to their positive impacts on vegetative growth and the availability of macro- and micronutrients reflecting the highest fruit content yield. These results are in harmony with those of **El-Khawaga and Maklad (2016)** on *Valencia orange*; they reported that adding N, K biofertilizers increased the yield of the tree from 6.6% to 18.32% with different biofertilizer sources, whereas using chemical fertilizers alone gave a lower effect for the same traits and growth parameters.

Our results also show that fruit quality improves when biofertilizers are combined with chemical source applications. In contrast, the control treatment displays the lowest fruit diameters, size, peel thickness, and juice volume / fruit compared to other treatments. The chemical fruit parameters, such as TSS value, were also increased using biofertilizers (T2 and T8) compared with the control. Meanwhile, the acidity percentage decreased by 51.9% and 34.7% of the control treatment in T8 and T7 in the two seasons. These findings agree with those reorted by **Eman et al. (2008)** on Washington Navel oranges, who stated that applying 50% mineral N + 50% organic N + biofertilizer is a promising treatment for improving yield and fruit quality. Furthermore, **Ennap (2016)** recommended applying 50% NPK + 55% farmyard + biofertilizer (*Azotobacter* + *Azospirillum* + *Bacillus circulans*) to Eureka lemon growers, as it results in the highest growth, yield, and fruit quality and enhances the nutritional state of the lemon trees. Many studies describe how biofertilizer application enhances citrus fruit quality by promoting phytohormone production **(Richardson et al. 2009; Zayan et al. 2016; Elavarasi et al. 2020; Khairi and Joody, 2025**), including auxins, gibberellins, cytokinins, IAA, and ethylene. Therefore, they play an important role in improving the growth of citrus trees and increasing both yield and fruit quality by stimulating cell division and enlargement, which positively affects yield quantity and fruit quality **(Ali et al. 2009; Spaepen et al. 2009; Ennab, 2016; Akalaa et al., 2022).**

Biofertilizers also positively influence the leaves nutrients content, including macro and micro elements, by enhancing the uptake and availability of these elements in the soil. Additionally, biofertilizers significantly increase the activity of soil enzymes such as nitrogenase, phenol oxidase, dehydrogenase, and phosphatase. As a result, they can enhance soil fertility and boost plants' resistance to environmental stresses **(Khairi and Joody, 2025)**, mainly promoting plant growth, fruit yield, and quality. Our results align with those of **(Aiman et al. 2009; Shamseldin et al. 2010; Xiao et al. 2014; El-Badawy et al. 2017; Hameed et al. 2018; Rana et al. 2020; Fang et al. 2020.Fikry et al 2020; Abbas and Abdulhakim, 2024; Shaaban and Abdel-Ati, 2025).**

5. Conclusions

The study indicated that adding *Azotobacter chroococcum* (Az14) as nitrogen fixing bacteria, and *Bacillus circulans* (B4) as potassium-dissolving bacteria to the Valencia orange trees growing under sandy soil conditions, succeeded in minimizing N mineral fertilizer requirements to 50-75 % of its recommended dose, while keeping most of the vegetative, yield, and fruit quality parameters at satisfactory levels compared with using chemical N fertilizer alone in the control treatment.

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

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