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Biogenic Nano-Fertilizers as a Sustainable Approach to Alleviate Nitrate Accumulation and Enrich Quality Traits of Vegetable Crops

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Abstract: Vegetables accumulate considerable amounts of nitrates that enter the human body through nutrition, causing severe problems. This study aims to determine celery plants' response to replacing mineral nitrogen fertilizers with bio-nanoparticles. Three different treatments of nano bio-nitrogen fertilizer (20, 30, and 40 ppm) in addition to traditional nitrogen (NH_4NO_3) treatment (100 kg N/acre) were applied on two celery cultivars (Balady and Utah Tall 52–75). Plant growth parameters, vitamin C, carotenoids, nitrate accumulation, macro-nutrient uptakes, and antioxidant activities were determined at the vegetative marketing stage. Our findings reveal a significant positive impact of replacing conventional nitrogen fertilizers with bio-nano-synthesized forms. Notably, applying bio-nanoparticles improved celery yield efficiency, ranging from 5.1 to 5.8 tons per acre, suggesting a viable alternative to traditional fertilization methods. Furthermore, transitioning from mineral to organic fertilizers in nanoparticle form reduced nitrate accumulation in fresh celery crops, decreasing nitrate levels from 342.5 ppm to as low as 100 ppm. This environmentally conscious approach offers a sustainable solution to mitigate chemical residues and enhance celery's flavor, nutritional value, and health benefits. Specifically, our results demonstrate alleviated nitrate contents in fresh celery leaves after applying bio-nano-fertilizer. Nitrate levels in treated plants decreased by up to 70.0% compared to traditional fertilization methods. This highlights the potential of organic nano-fertilizers to address concerns related to nitrate accumulation, thereby promoting safer and healthier vegetable consumption. By advocating for organic nano-fertilizers, we propose a promising strategy to optimize celery fertilizing management, ensuring sustainable farming and consumer well-being.

Keywords: organic farming; bio-nano-fertilizers; vegetables; nitrate; food security

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1. Introduction

Traditional fertilizers (TF) have negative environmental impacts, damaging aquatic systems and reducing soil fertility and crop productivity [1]. This is due to the fact that TF has poor efficacy as crops utilize less than 20% of the applied fertilizers, and the other percentage is leached to the surrounding agro-ecosystem, leading to environmental deterioration, eutrophication, soil quality degradation, increased GHG emissions, and causing negative impacts on human health [2]. Nitrogen is a primary nutrient inorganic fertilizer and is a critical requirement for crop growth and development [3]. Nitrogen is an essential macro-nutrient for plants, playing a pivotal role in forming amino acids, which serve as the fundamental building blocks of enzymes and proteins crucial for plant function [4,5]. Furthermore, nitrogen is an integral component of the chlorophyll molecule, essential for photosynthesis, facilitating the absorption of sunlight energy to promote robust plant growth and maximize grain yield [6]. Excessive nitrogen fertilization may cause high crop nitrate accumulation, negatively affecting raw and minimally prepared vegetables'

morphology, safety, nutritional quality, and shelf life [7,8]. Nitrogen is the most critical nutrient that plants need because it plays an influential role in achieving high grain and protein yields when the appropriate balance between nitrogen nutrients is achieved, as excess and nitrogen deficiency have adverse effects on crop growth and development [9]. An increase in harmful forms of nitrogen (nitrate, ammonia, and nitrogen oxides) in the soil leads to environmental damage, such as air pollution, promoting global climate change, eutrophication, and soil acidification [10,11].

Nitrates are formed as a portion of the nitrogen cycle and play an essential function during the growth and development of plants. Due to their cumulative properties, nitrates are crucial in vegetables [12–14]. Vegetables accumulate a significant amount of nitrates [15] from nitrogen-based fertilizers, which boost plant growth and development [16]. Leafy vegetables contain a substantial proportion of nitrate [17]. Vegetables comprise 80% of dietary nitrate consumption, meaning human body exposure to nitrate is mainly associated with vegetable intake [18]. Nitrates are inert and have biological activities only with nitrate reduction to nitrite and then serving as a reactant with amines and/or amides in forming N-nitroso [19] and various other nitrogen compounds that have toxicological severe impacts on human health, and this gives nitrate special attention.

The application of nanotechnology in agriculture was increasing in recent years and constitutes a valuable tool to achieve the goal of sustainable food production all over the world [20,21]. Nanotechnology, which is of great interest in the agricultural revolution, enhanced bioavailability and bioactivity, adherence effects, and surface effects of nanoparticles [22,23]. These unique properties are due to the small molecular size and modified intermolecular interactions. Among the state-of-the-art nano-enabled agricultural technologies, nano bio-fertilizers stand out as an innovative solution for enhancing vegetable productivity and quality [24]. These fertilizers aim to address issues associated with traditional fertilizers by providing more efficient nutrient delivery. This technology represents a smart tool for sustainability, reducing environmental impact and increasing yields and nutritional value [25]. Biogenic nano-fertilizers were studied to increase nutrient efficiency and improve plant nutrition compared to conventional fertilizers. Bio-nano-fertilizers (Bio-NPs) are made from microbes and can enhance yields by up to 25%. They can also help with nutrient management by boosting nutrient uptake.

Bio-NPs can mitigate environmental ecotoxicity and generate a sustainable agricultural ecosystem [26]. This novel approach to sustainable agriculture can potentially decrease costs and increase productivity. Bio-NP usage has various benefits, such as enhanced plant growth and reduced soil leaching and pollution. The excessive use of synthetic fertilizers contributes to pollution and degrades soil and water quality. Moreover, the runoff of chemicals releases greenhouse gases [27]. Bio-NPs will boost crop yield and contribute to nutritional health, food security, and environmental quality [28].

Regarding those mentioned above, this study hypothesizes that replacing traditional mineral nitrogen fertilizers with bio-nano-fertilizers will significantly reduce nitrate accumulation in vegetables and enhance their nutritional quality and yield. Bio-nano-fertilizers are expected to provide more efficient nutrient delivery, leading to improved plant growth parameters and reduce environmental impact compared to TF, leading to promoting both sustainable farming practices and consumer health.

2. Materials and Methods

2.1. The Experimental Conditions

A field experiment was conducted at the Nubariya Experimental Station at the National Research Center in Beheira Governorate, Egypt, during two consecutive growing seasons from 2021 to 2023. Firstly, a mixture of 1.5 kg calcium superphosphate ($CaH_4P_2O_8$) plus 5 kg potassium sulfate (K_2SO_4) per 150 m^2 of the land area was applied during soil preparation. Celery was selected as a model plant in this experiment. Celery (*Apium graveolens*) is a leafy vegetable crop that belongs to the Apiaceae family and is native to the Mediterranean region [29], valued in the diet for its high nutritional value [30]. Seedlings

of two celery cultivars (local Egyptian cv "Balady" and imported cv "Utah Tall 52–75") were sown at 50 cm between rows and 25 cm between plants. Nitrogen mineral fertilizer (100 kg N/acre) and biogenic nano-fertilizer (Bio-NPs) were applied as organic nitrogen sources. Bio-NP concentrations (20, 30, and 40 ppm) were sprayed on celery plants three times (15, 30, and 45 days after transplanting), while traditional mineral nitrogen fertilizer was split into two equal doses and added at 30 and 45 days after transplanting.

Samples from the soil of the experiment were taken before cultivation and subjected to physical and chemical analysis. The soil analysis results are shown in Table 1. The mechanical analysis indicated clearly that the texture of the soil is sandy loam. The soil pH ranges from 8.1 to 8.3 across the different depths, indicating alkaline conditions. Electrical conductivity (EC) values increase with depth, ranging from 4.42 to 7.85 dSm^{-1}, suggesting a higher concentration of dissolved salts in the deeper layers. Organic matter (OM) content remains very low, ranging from 0.3% to 0.4%.

Table 1. Physical properties and chemical analysis of the soil taken from the experimental site.

Physical Properties				Soil Depth (cm)	Chemical Properties							
Texture	Sand%	Silt%	Clay%		pH	EC, dSm^{-1}	OM, %	HCO_3	Cl	Na	K	Ca
								meq/L				
Sandy-loam	54.28	30.0	14.94	0–25	8.1	4.42	0.4	3.1	4.0	8.5	1.3	1.0
	55.19	29.59	13.43	25–50	8.1	5.13	0.3	0.4	3.4	6.8	1.1	1.8
	57.71	30.92	11.37	50–75	8.3	7.85	0.3	2.0	6.2	8.0	0.6	2.2

The low concentration of organic matter (OM) observed in the soil analysis indicates a deficiency in organic material, highlighting the need for nitrogen fertilization. The concentrations of bicarbonate (HCO_3), chloride (Cl), sodium (Na), potassium (K), and calcium (Ca) vary across the different depths.

2.2. Nanoparticles Synthesis

Aspergillus tubingensis TFR-29 was grown in a 500 mL flask containing 250 mL P.D. broth. The pH of the medium was adjusted to 5.8, and the entire culture was subjected to continuous shaking on a rotary shaker (75 rpm) at 28 °C for three days (72 h). After complete incubation, mycelial fungal balls were separated from the culture mixture by filtration using Whatman (Whatman, Maidstone, UK) No. 42 filter paper, and the mycelial fungi were washed three times with sterile double distilled water. Harvested fungi were resuspended in sterile Milli-Q water (per 15 g of mycelium in a 500 mL conical flask) and placed again in a rotary shaker (75 rpm) at 28 °C for 48 h. After incubation, the desired cell-free filtrate was obtained by separating the fungal biomass using Whatman filter paper. Using the required cell-free filter, aqueous ammonium nitrate saline (Technogene, Cairo, Egypt) at a final concentration of 0.1 mM, 0.5 mM, and 1 mM was prepared in Erlenmeyer flasks as a preliminary experiment. A salt concentration of 0.5 mM was found to be optimal for the synthesis of monodisperse nitrogen nanoparticles. The mixture was kept on a rotary shaker at 28 °C at 75 rpm. The reaction was allowed to proceed, and the bioconverted product was collected periodically for particle size characterization using a particle size analyzer [31].

2.3. Nanoparticles Measurement Techniques

Transmission electron microscopy (TEM): A transmission electron microscope (JEM-1234, Hitachi High-technologies, Tokyo, Japan) with a 120 KV operating voltage, a 600,000× magnification power, a 0.3 nm resolving power, a CCD camera, and a programmed heating/cooling facility with a temperature range of −190 °C to 1000 °C on a copper grid coated with carbon were used to examine the samples. The size of NH_4NO_3 nanoparticles was investigated using transmission electron microscopy (TEM), which in-

dicated the existence of spherical vesicles with diameters ranging from 2.97 to 12.13 nm (Figure 1).

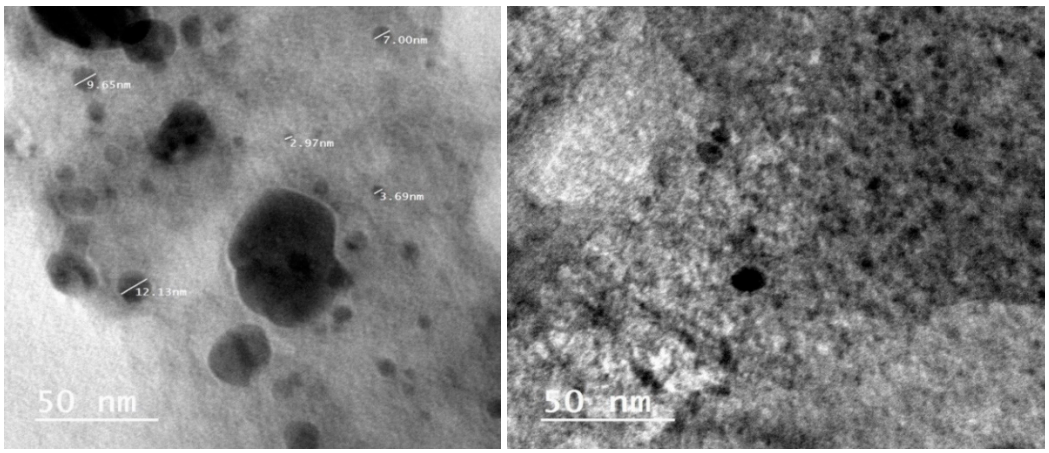


Figure 1. Transmission electron microscopy of NH_4NO_3 nanoparticles.

Scanning electron microscopy: Micrographs of the samples were taken using the Hitchi-S-3400N SEM instrument (Quantum FEG 250, FEI, Brno, Czech Republic). The instrument was operated at an accelerating voltage of 30 kV. SEM micrographs of biologically synthesized nanoparticles were obtained by ultracentrifugation of the suspension containing nanoparticles, and the pellets were washed with phosphate buffer added 0.25% glutaraldehyde (prepared in sodium phosphate buffer pH 7.2), incubated overnight at room temperature under vacuum desiccator, then rinsed with sodium phosphate buffer at pH 7.2 (Figure 2).

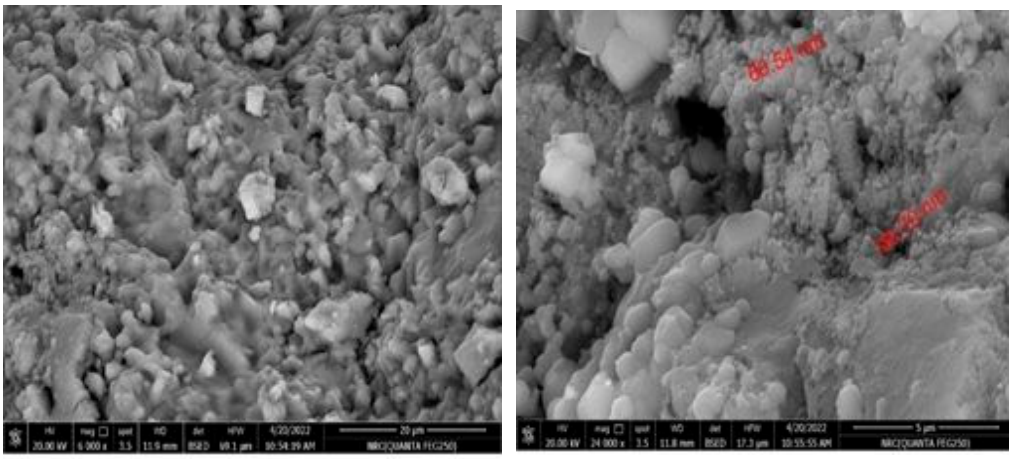


Figure 2. Scanning electron microscopy (SEM) of NH_4NO_3 nanoparticles.

The samples were dehydrated by passing through an E.M. grade ethanol series consisting of 30, 50, 70, 80, 90, and 100% ethanol, each incubated for 10 min. Samples were placed on aluminum stubs using a double-sided carbon tap—sputter coated with heavy metal and gold. Samples were kept in the chamber of an SEM instrument, and micrographs of each sample were obtained at various magnifications at different electron modes [32].

FTIR spectroscopy: The surface composition and functionalization of nanoparticles were assessed using FTIR spectroscopy analysis. The KBr medium facilitates the generation of sampling FTIR spectra, enabling data collection between 400 and 4000 cm. The presence of functional groups on the surface of the bio-NH_4NO_3 nanoparticles was confirmed using the Fourier transform infrared spectroscopy (FTIR) technique. Figure 3 displays the spectrum of NH_4NO_3 nanoparticles. The functional groups found in the generated NH_4NO_3

nanoparticles are represented by the peaks in the spectra [33]. The absorption peaks of the samples are presumed to be located within the range of 3861.11, 3731.64, 3178.60, 2937.90, 1788.16, 1762.19, 1632.46, 1351.67, 982.84, 540.11, and 407.22 cm^{-1}. The stretching vibration of the O-H bond and the stretching vibration of the C-H bond, respectively, may be responsible for the observed peaks at 3861.11 cm^{-1} and 3731.64 cm^{-1}.

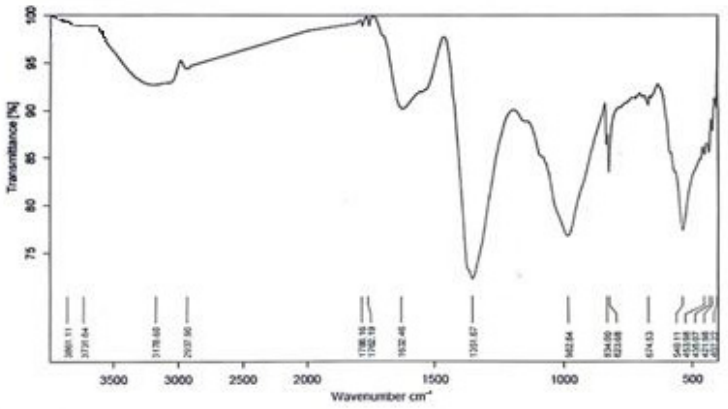


Figure 3. FTIR Spectroscopy of NH_4NO_3 nanoparticles.

The absorption peak at 407.22 cm^{-1} is attributed to the vibration pattern of the stretching vibrations of the metal–oxygen (NH_4NO_3) system. The observed peaks at 982.84 cm^{-1} and 540.11 cm^{-1} can be attributed to the in-plane bending or vibration of primary and secondary alcohols, respectively. The observed peak at 1351.67 cm^{-1} can be ascribed to the vibrational mechanisms displayed by aromatic nitro compounds and alkyl groups. The extracellular extracts of Saccharomyces cerevisiae encompass various functional categories, namely protein, alcohol, phenolic group, carbohydrates, and fatty acids. Extracellular proteins can create a protective layer that can hinder the aggregation of nanoparticles and improve their stability [34].

2.4. Plant Growth Measurements

Random samples of five plants were taken after 105 days of cultivation (marketing stage). Plant height (cm), number of leaves per plant (Pcs), fresh and dry plant weights (g/plant), and fresh and dry yield per acre were registered.

2.5. Photosynthetic Pigments

Chlorophylls and carotene were estimated from fresh celery leaves. The leaves were collected, washed with distilled water, and wiped to remove moisture. A total of 0.2 g of fresh celery leaves were grinded in 5 mL acetone 90%. Absorption (Abs) was taken using a spectrophotometer (zhimadzm UV-240, Labexchange—Burladingen, Germany) with wavelength (663, 644, and 452.5 nm) for chlorophyll a, chlorophyll b, and carotenoids, respectively. The results are expressed in mg/g FW, as described in previous works [4,35,36] using the following equations:

$$\text{Chlorophyll a (Chl. a)} = 10.3 \times \text{Abs663} - 0.918 \times \text{Abs644}$$

$$\text{Chlorophyll b (Chl. b)} = 9.7 \times \text{Abs644} - 3.87 \times \text{Abs663}$$

$$\text{Carotene} = 4.2 \times \text{Abs452.5} - (0.0264 \times \text{Chl. a} + 0.4260 \times \text{Chl. b}).$$

2.6. Vitamin C and Nitrate Contents

Vitamin C (mg/100 g) in fresh leaves was measured using a spectrophotometer (zhimadzm UV-240, Labexchange—Burladingen, Germany) at a wavelength of 261 nm [37]. Nitrate contents were measured using "Merckoquant" NO_3 testing strips (E. Merck, Darm-

stadt, Germany) on lawn sap for zinc determination. Sap was prepared from the celery stem [38–40].

2.7. Macro-Nutrient Contents

Samples of celery leaves were well-dried to a constant weight. The samples were ground separately; then, samples were attained and acid-digested. The percentages of nitrogen, phosphorus, potassium, and calcium in the acid digestion samples were determined [41].

2.8. Determination of Total Phenolic, Total Flavonoids, and Antioxidant Capacity

Total phenolic: Phenolic was extracted by ethanol 80%. Phenolic were estimated by adding 1 mL of sample and 70 mL distilled water followed by Folin–Ciocalteau reagent and 15 mL of saturated sodium carbonate solution, incubated at room temperature for 30 min and measured at 765 nm in a spectrophotometer [42].

Total flavonoid: The total flavonoid content was estimated using a 2% aluminum chloride ($AlCl_3$) solution in methanol [43] to 1.5 mL extract (1:5 diluted), and 1.5 mL $AlCl_3$ in methanol was added. The samples were incubated at 30 °C for 10 min. The absorbance was recorded at 368 nm.

Antioxidant assays: Extracts' free radical scavenging capacity was determined using the stable DPPH [44]. Aliquot 50 µL of extracts were mixed with 2.95 mL of 200 µM of 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm against pure methanol after one hour of incubation in the dark. The standard curve was prepared using Trolox. Results are expressed as milligrams of trolox equivalent (mg T.E.) per gram of celery sample [45].

Antioxidant activities were measured using the $ABTS^+$ method. 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was dissolved in distilled water at a concentration of 7 µM. The $ABTS^+$ radical effect was obtained by reacting the ABTS solution with 2.45 mM potassium sulfate and then keeping it at room temperature in the dark for 16 h. To follow the infusion, the resulting $ABTS^+$ solution was diluted with distilled water to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. A blank reading was taken in the detector. After adding 3.0 mL of diluted $ABTS^+$ solution (A734 nm = 0.70 ± 0.02) to 30 µL of extracts, the absorbance was measured after 6 min of mixing. Results were corrected for dilution and expressed in µm T.E. per 100 g dry weight [46].

2.9. Statistical Analyses

The experiment consists of ten treatments representing the interaction between two cultivars and four fertilizer treatments. A split plot design with three replicates was adopted in this study. Two-way ANOVA analyses were performed using CO-stat software V. 6.45. The data were expressed as means ± SD, and Tukey analysis for comparison means was performed.

3. Results

3.1. Plant Growth Traits

Data in Table 2 represent the effect of organic replacement of mineral nitrogen fertilization on vegetative growth parameters of two celery cultivars (Balady and Utah Tall 52–75) during two growing seasons (2021–2022 and 2022–2023). The differences between the two cultivars were highly significant in all stages in both investigated seasons. The results show that "Utah Tall 52–75" plants treated with concentrations of 30 and 40 ppm bio nanoparticles (Bio-NPs) recorded the highest value for plant height, with an increase of 16.8% and 16.4% compared to the control plants in the first season, while plant height was decreased in the second season with an increased rate of 10.0% compared to control.

Table 2. Effect of biogenic nanoparticles (20, 30, and 40 ppm) on the vegetative parameters of two celery plants (Balady and Tall Utah 52–75) compared to traditional nitrogen fertilizer (100 kg).

Cultivars	Nitrogen Source and Concentration	First Season			
		Plant Height (cm)	Number of Leaves/Plant	Fresh Weight (g/plant)	Dry Weight (g/plant)
Local Egyptian Balady	Mineral 100 kg	55.3 ± 0.24 cd	73.3 ± 2.72 e	566.3 ± 13.44 d	54.4 ± 0.62 d
	Bio-NPs 20 ppm	56.2 ± 0.21 c	73.9 ± 4.02 e	509.4 ± 9.41 f	48.9 ± 0.51 f
	Bio-NPs 30 ppm	59.6 ± 0.17 bc	82.6 ± 3.92 cd	560.3 ± 11.27 d	53.1 ± 0.68 de
	Bio-NPs 40 ppm	61.1 ± 0.19 b	82.7 ± 3.04 cd	520.5 ± 13.51 e	50.6 ± 0.41 e
Tall Utah 52–75	Mineral 100 kg	58.1 ± 0.26 c	99.1 ± 2.02 ab	1122.4 ± 11.03 a	83.9 ± 53 b
	Bio-NPs 20 ppm	67.9 ± 0.21 ab	98.8 ± 2.13 ab	989.5 ± 8.93 c	83.2 ± 0.74 b
	Bio-NPs 30 ppm	69.8 ± 0.32 a	102.2 ± 2.92 a	1121.4 ± 11.12 a	83.2 ± 1.04 a
	Bio-NPs 40 ppm	69.5 ± 0.22 a	99.2 ± 2.01 a	1014.4 ± 9.38 b	82.5 ± 0.92 a
Second season					
Local Egyptian Balady	Mineral 100 kg	44.3 ± 04.7 d	84.8 ± 2.54 e	579.3 ± 3.28 d	54.8 ± 0.73 d
	Bio-NPs 20 ppm	53.3 ± 0.37 c	82.7 ± 1.09 ef	529.5 ± 7.28 de	50.4 ± 1.02 e
	Bio-NPs 30 ppm	54.2 ± 0.35 bc	87.6 ± 2.38 d	572.1 ± 9.26 bc	53.9 ± 0.94 de
	Bio-NPs 40 ppm	56.9 ± 0.41 b	88.1 ± 2.15 cd	556.9 ± 7.37 de	52.6 ± 0.61 de
Tall Utah 52–75	Mineral 100 kg	53.9 ± 0.33 c	99.9 ± 3.01 bc	1018.3 ± 8.42 c	95.6 ± 1.05 ab
	Bio-NPs 20 ppm	54.3 ± 0.27 bc	98.4 ± 2.02 bc	1008.2 ± 7.32 c	93.2 ± 0.83 b
	Bio-NPs 30 ppm	59.8 ± 0.49 a	107.1 ± 2.84 a	1116.5 ± 8.53 a	95.1 ± 0.62 a
	Bio-NPs 40 ppm	59.9 ± 0.52 a	106.7 ± 2.94 a	1013.5 ± 6.29 b	93.8 ± 0.81 b

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

The Egyptian Balady cultivar gave the maximum plant height (61.1 cm) when the maximum dose of Bio-NPs (40 ppm) was sprayed with an increase rate of 10% compared to the control plants in the first season, while the highest value recorded in the second season (more than 56.9 cm) with an increase rate of 22.1% compared to control (N mineral). The lowest plant value was obtained with mineral treatment.

Table 2 shows a significant impact due to the interaction between celery cultivars and fertilization treatments. The highest number of leaves per plant (NLP) was obtained from Utah Tall 52–75 plants sprayed with 30 and 40 ppm Bio-NPs. The NLP in Utah Tall 52–75 plants increased by 10.0% in the first season and 17% in the second season, while the NLP increasing rate in the Egyptian cultivar was 5% in the first season and 3.5% in the second season compared to control plants which were fertilized by mineral nitrogen (100 kg/acre).

Data in Table 2 illustrate the response of celery cultivars to complete organic replacement of mineral nitrogen fertilization on plant fresh weight (FW) and plant dry weight (DW) in the vegetative marketing stages of celery cultivars. The data show that the highest values of FW and DW were recorded with control and Bio-NPs–30 ppm in Utah Tall 52–75 plants where the plant reached more than (1.12 kg FW and 83 g DW) in the first season and (1.11 kg FW and 95 g DW) in the second season and no statistically significant differences were recorded between the two treatments. In Balady plants, control and Bio-NPs–30 ppm treatment gave the maximum weights, as the highest weight value was recorded during the two study seasons (566.3, 560.3, and 579.3, 572.1 g FW) and (54.4, 53.1 and 54.8, 53.9 g DW), in first and second seasons, respectively. Meanwhile, Bio-NPs-20 ppm registered the lowest FW and DW.

3.2. Photosynthetic Pigments

Table 3 showed a significant impact due to the interaction between celery cultivars and fertilization treatments. The highest chlorophyll a and b and total chlorophyll values were obtained from the Balady plants, fertilized with mineral nitrogen, and 30 and 40 ppm

nano-biofertilization. Egyptian cv plants decreased by 8% in the first season and 3% in the second season, while the rate of increase in the treated plants of the Tall 52–75 cv was 7% in the first season and 3.0% in the second season compared to the plants, which were fertilized by Bio-NPs nitrogen (control). The highest value of carotenoids was obtained from Local Egyptian Balady cv (0.47 and 0.45 mg/g) with fertilizing by 30 ppm nano-biofertilization in both seasons. The study results indicate significant chlorophyll and carotenoid level variations among two celery genotypes, Local Egyptian Balady and Tall Utah 52–75, under different nitrogen sources and concentrations across two seasons. For the Local Egyptian Balady genotype, the highest chlorophyll levels were observed under the mineral nitrogen source (100 kg) in both seasons, with values of 1.58 mg/g (chlorophyll a), 0.66 mg/g (chlorophyll b), and 2.25 mg/g (total chlorophyll) in the first season.

Table 3. Effect of biogenic nanoparticles (20, 30, and 40 ppm) on photosynthetic pigments (Chl. a, Chl. b, and Carotene) of two celery plants (Balady and Tall Utah 52–75) compared to traditional nitrogen fertilizer (100 kg).

Genotype	Nitrogen Source and Concentration	First Season			
		Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
Local Egyptian Balady	Mineral 100 kg	1.58 ± 0.04 a	0.66 ± 0.01 a	2.25 ± 0.01 a	0.42 ± 0.02 c
	Bio-NPs 20 ppm	1.41 ± 0.03 b	0.61 ± 0.01 b	2.03 ± 0.02 d	0.44 ± 0.02 b
	Bio-NPs 30 ppm	1.36 ± 0.02 c	0.59 ± 0.03 c	1.97 ± 0.01 a	0.47 ± 0.01 a
	Bio-NPs 40 ppm	1.33 ± 0.02 d	0.59 ± 0.01 d	1.92 ± 0.01 c	0.45 ± 0.03 b
Tall Utah 52–75	Mineral 100 kg	1.34 ± 0.03 cd	0.61 ± 0.02 b	1.96 ± 0.01 d	0.41 ± 0.02 c
	Bio-NPs 20 ppm	1.28 ± 0.01 d	0.57 ± 0.01 d	1.87 ± 0.03 c	0.39 ± 0.02 d
	Bio-NPs 30 ppm	1.18 ± 0.02 e	0.51 ± 0.01 fe	1.70 ± 0.01 c	0.39 ± 0.01 d
	Bio-NPs 40 ppm	1.21 ± 0.01 de	0.55 ± 0.03 de	1.75 ± 0.01 bc	0.40 ± 0.02 cd
		Second season			
Local Egyptian Balady	Mineral 100 kg	1.52 ± 0.02 a	0.75 ± 0.03 a	2.29 ± 0.02 a	0.49 ± 0.02 a
	Bio-NPs 20 ppm	1.48 ± 0.02 b	0.73 ± 0.01 b	2.23 ± 0.01 b	0.43 ± 0.01 c
	Bio-NPs 30 ppm	1.32 ± 0.01 c	0.69 ± 0.02 c	2.03 ± 0.01 d	0.45 ± 0.02 b
	Bio-NPs 40 ppm	1.42 ± 0.02 d	0.58 ± 0.01 cd	2.00 ± 0.01 b	0.43 ± 0.02 c
Tall Utah 52–75	Mineral 100 kg	1.51 ± 0.01 a	0.47 ± 0.01 d	1.98 ± 0.01 d	0.44 ± 0.01 b
	Bio-NPs 20 ppm	1.38 ± 0.03 bc	0.51 ± 0.01 e	1.91 ± 0.02 cd	0.38 ± 0.02 d
	Bio-NPs 30 ppm	1.25 ± 0.02 d	0.51 ± 0.02 e	1.76 ± 0.01 c	0.39 ± 0.02 d
	Bio-NPs 40 ppm	1.41 ± 0.02 c	0.48 ± 0.01 cd	1.90 ± 0.02 d	0.36 ± 0.02 e

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

Conversely, the lowest chlorophyll levels were recorded under Bio-NPs at 40 ppm, indicating decreased chlorophyll content with increasing Bio-NPs concentrations. Similar trends were observed for the Tall Utah 52–75 genotype, with the highest chlorophyll levels under mineral nitrogen (100 kg) and decreasing levels with increasing Bio-NP concentrations. In the second season, chlorophyll levels remained highest under mineral nitrogen for both genotypes, while decreasing concentrations of Bio-NPs led to lower chlorophyll levels. Overall, the results suggest that mineral nitrogen resulted in higher chlorophyll levels than Bio-NPs at various concentrations for both genotypes and seasons, indicating the importance of nitrogen source and concentration in regulating plant chlorophyll synthesis.

The carotenoid levels in the studied plant genotypes, Local Egyptian Balady and Tall Utah 52–75, showed similar trends to chlorophyll levels across different nitrogen treatments and concentrations in both seasons. In the first season, the highest carotenoid levels for the Local Egyptian Balady genotype were recorded under the mineral nitrogen source (100 kg), with 0.42 mg/g values. Conversely, the lowest carotenoid levels were observed

under Bio-NPs at 30 ppm, indicating decreased carotenoid content with increasing Bio-NPs concentrations. Similar trends were observed for the Tall Utah 52–75 genotype, with the highest carotenoid levels under mineral nitrogen (100 kg) and decreasing levels with increasing Bio-NP concentrations. In the second season, carotenoid levels remained highest under mineral nitrogen for both genotypes, while decreasing concentrations of Bio-NPs led to lower carotenoid levels. Overall, the results suggest that mineral nitrogen resulted in higher carotenoid levels compared to Bio-NPs at various concentrations for both genotypes and seasons, indicating the importance of nitrogen source and concentration in regulating carotenoid synthesis in plants.

3.3. Yield and Its Quality

Table 4 illustrates the response of celery cultivars to organic replacement of mineral nitrogen fertilization on fresh and dry yield of two celery cultivars during the two growing seasons. The results show that Utah Tall 52–75 cv. registered higher values of the fresh yield and dry yield compared to Balady. The differences between the two cultivars were highly significant in both seasons. Data in Table 4 reveal that the highest fresh yield (11.2 and 11.7 ton/acre was obtained from Utah Tall 52–75 cv plants when Bio-NPs 30 ppm was applied. The lowest fresh weight was obtained from the Balady cultivar when it was applied with Bio-NP 20 ppm.

Table 4. Effect of biogenic nanoparticles (20, 30, and 40 ppm) on yield and quality attributes of two celery plants (Balady and Tall Utah 52–75) compared to traditional nitrogen fertilizer (100 kg).

Genotype	Nitrogen Source and Concentration	First Season			
		Fresh Yield (ton/Acre)	Dry Yield (Kg/Acre)	Vitamin C Content (mg/100 g)	NO_3–N ppm
Local Egyptian Balady	Mineral 100 kg	5.1 ± 0.03 e	521.3 ± 5.27 g	5.1 ± 0.03 c	342.5 ± 9.04 a
	Bio-NPs 20 ppm	4.2 ± 0.01 f	523.6 ± 4.84 g	5.3 ± 0.02 b	237.9 ± 7.37 c
	Bio-NPs 30 ppm	5.2 ± 0.06 e	571.9 ± 6.04 fg	5.6 ± 0.02 a	132.1 ± 9.25 de
	Bio-NPs 40 ppm	5.1 ± 0.02 e	563.7 ± 3.02 fg	5.4 ± 0.05 ab	174.3 ± 5.93 d
Tall Utah 52–75	Mineral 100 kg	8.5 ± 0.02 c	783.5 ± 7.24 ef	5.1 ± 0.03 c	311.2 ± 8.59 b
	Bio-NPs 20 ppm	10.3 ± 0.03 b	924.1 ± 6.73 c	5.3 ± 0.01 b	121.5 ± 7.47 e
	Bio-NPs 30 ppm	11.2 ± 0.02 a	1002.5 ± 7.14 a	5.6 ± 0.02 a	108.5 ± 8.35 f
	Bio-NPs 40 ppm	10.4 ± 0.02 ab	982.4 ± 5.93 b	5.4 ± 0.03 ab	116.4 ± 7.28 f
Second season					
Local Egyptian Balady	Mineral 100 kg	5.7 ± 0.06 de	592.4 ± 7.04 d	5.2 ± 0.05 bc	378.3 ± 6.84 a
	Bio-NPs 20 ppm	5.2 ± 0.04 e	567.7 ± 9.32 e	5.2 ± 0.03 bc	243.6 ± 5.93 b
	Bio-NPs 30 ppm	6.1 ± 0.08 d	603.1 ± 11.32 d	5.4 ± 0.05 a	201.4 ± 7.37 c
	Bio-NPs 40 ppm	5.8 ± 0.03 de	556.2 ± 9.73 f	5.1 ± 0.03 c	198.5 ± 9.46 c
Tall Utah 52–75	Mineral 100 kg	9.7 ± 0.05 b	893.5 ± 8.04 cd	5.2 ± 0.03 bc	312.4 ± 11.04 a
	Bio-NPs 20 ppm	9.2 ± 0.02 bc	1018.3 ± 9.47 bc	5.2 ± 0.04 bc	213.6 ± 8.64 bc
	Bio-NPs 30 ppm	11.7 ± 0.03 a	1173.8 ± 7.62 a	5.3 ± 0.02 ab	187.4 ± 9.27 d
	Bio-NPs 40 ppm	11.3 ± 0.02 a	1043.6 ± 9.51 b	5.2 ± 0.05 bc	99.2 ± 7.47 d

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

The results show that Utah Tall 52–75 with Bio-NPs 30 ppm recorded the highest value of vitamin C content (5.6 and 5.4 mg/100 g) compared to Balady cv. The differences between the two cultivars were highly significant in both seasons. The nitrate percentage increased in the plants treated with traditional nitrogen fertilizer, and plants treated with Bio-NPs at 30 ppm recorded the lowest percentage of nitrate in the leaves with a decrease rate of up to 35%. This is because organic fertilization does not reduce the accumulation of nitrates.

In the first season, when comparing the mineral nitrogen treatment (100 kg) to the various Bio-NPs treatments, notable differences in fresh yield, dry yield, vitamin C content, and NO_3 –N ppm levels were observed across both genotypes. The vegetative yield for the Local Egyptian Balady genotype ranged from 5.1 to 5.7 tons/Acre under mineral nitrogen (100 kg) and Bio-NPs treatments, respectively. Similarly, the dry yield varied from 521.3 to 592.4 kg/acre, with the highest yield observed under the mineral nitrogen treatment (100 kg). Additionally, the NO_3 –N ppm levels were consistently lower in the Bio-NPs treatments compared to mineral nitrogen (100 kg).

In the second season, the vegetative yield for the Local Egyptian Balady genotype ranged from 5.2 to 6.1 ton/acre, with the highest yield observed under Bio-NPs at 30 ppm and the lowest under Bio-NPs at 20 ppm. Similarly, for the Tall Utah 52–75 genotype, the fresh yield varied from 9.2 to 11.7 ton/acre, with the highest yield recorded under Bio-NPs at 30 ppm and the lowest under mineral nitrogen (100 kg). Regarding dry yield, the Local Egyptian Balady genotype produced between 556.2 and 603.1 kg/acre, with the highest yield under Bio-NPs at 30 ppm and the lowest under Bio-NPs at 20 ppm. Meanwhile, the Tall Utah 52–75 genotype yielded between 893.5 and 1173.8 kg/acre, with the highest yield under Bio-NPs at 30 ppm and the lowest under mineral nitrogen (100 kg). Regarding vitamin C content, the Local Egyptian Balady genotype exhibited levels ranging from 5.1 to 5.4 mg/100 g, with the highest content observed under Bio-NPs at 30 ppm and the lowest under mineral nitrogen (100 kg).

Similarly, for the Tall Utah 52–75 genotype, the vitamin C content varied from 5.2 to 5.3 mg/100 g, with the highest content under Bio-NPs at 30 ppm and the lowest under mineral nitrogen (100 kg). NO_3 –N ppm levels also showed consistent trends, with lower concentrations observed under higher doses of Bio-NPs than mineral nitrogen (100 kg). The Local Egyptian Balady genotype recorded levels ranging from 198.5 to 378.3 ppm, while the Tall Utah 52–75 genotype exhibited levels ranging from 99.2 to 312.4 ppm. Overall, these results underscore the potential of Bio-NPs in enhancing vegetative and dry yields, improving vitamin C content, and reducing nitrate accumulation in plants, thereby contributing to sustainable agricultural practices in both genotypes across different nitrogen treatments.

3.4. Macro-Nutrient Uptake

The results show significant differences regarding nitrogen content between the two cultivars for both seasons. The highest nitrogen percentage value was obtained from traditional mineral fertilization treatment (100 kg) in both seasons, followed by Bio-NPs-20 ppm nano-biofertilizer treatment. Meanwhile, the lowest nitrogen percentage was obtained from Bio-NPs-30 ppm nano-biofertilizer treatment, preceded by Bio-NPs-40 ppm nano-biofertilizer treatment.

Results also indicate a significant effect for the interaction between celery cultivars and fertilization treatments. Balady and Utah Tall 52–75 cv obtained the highest nitrogen percentage. Celery plants were fertilized with Bio-NPs-30 ppm nano-biofertilizer treatment, followed by plants sprayed with Bio-NPs-40 ppm. Meanwhile, the lowest nitrogen percentage was obtained from Balady cv. and Utah Tall 52–75 plants were fertilized by mineral fertilizer treatment, followed by Balady and Utah Tall 52–75 celery plants. Bio-NPs fertilized plants with 20 ppm nano-biofertilizer treatment in both seasons. There were no significant differences between the two cultivars for both seasons. Balady cv recorded a higher value of phosphorus percentage than Utah Tall 52–75 cv. According to the data in Table 5, the effect of the interaction and the highest phosphorus percentage was obtained from Balady plants fertilized with nano-biofertilizer Bio-NPs 30 ppm treatment (15.5%). Meanwhile, Utah Tall 52–75 cv obtained the lowest phosphorus percentage.

Table 5. Effect of biogenic nanoparticles (20, 30, and 40 ppm) on macro-nutrient uptake (nitrogen, phosphorus, potassium, and calcium) of the dried celery leaves (Balady and Tall Utah 52–75) compared to traditional nitrogen fertilizer (100 kg).

Genotype	Nitrogen Source and Concentration	First Season			
		Nitrogen, %	Phosphorus, %	Potassium, %	Calcium, %
Local Egyptian Balady	Mineral 100 kg	5.3 ± 0.02 a	0.5 ± 0.02 b	2.2 ± 0.02 c	2.1 ± 0.04 d
	Bio-NPs 20 ppm	4.1 ± 0.01 b	0.5 ± 0.01 b	2.4 ± 0.02 b	2.1 ± 0.07 d
	Bio-NPs 30 ppm	3.7 ± 0.02 c	0.6 ± 0.01 a	2.7 ± 0.01 a	2.3 ± 0.0 b
	Bio-NPs 40 ppm	3.6 ± 0.01 cd	0.6 ± 0.02 a	2.6 ± 0.02 ab	2.3 ± 0.03 b
Tall Utah 52–75	Mineral 100 kg	5.2 ± 0.02 a	0.45 ± 0.01 b	2.2 ± 0.02 c	2.2 ± 0.09 c
	Bio-NPs 20 ppm	4.1 ± 0.03 b	0.4 ± 0.02 c	2.6 ± 0.01 ab	2.3 ± 0.07 b
	Bio-NPs 30 ppm	2.1 ± 0.02 de	0.5 ± 0.02 b	2.6 ± 0.01 a	2.4 ± 0.05 a
	Bio-NPs 40 ppm	2.2 ± 0.02 d	0.5 ± 0.02 a	2.6 ± 0.02 ab	2.4 ± 0.08 a
Second season					
Local Egyptian Balady	Mineral 100 kg	5.1 ± 0.03 a	0.5 ± 0.01 b	2.2 ± 0.01 c	2.2 ± 0.04 c
	Bio-NPs 20 ppm	3.2 ± 0.02 bc	0.4 ± 0.01 c	2.4 ± 0.01 b	2.1 ± 0.03 d
	Bio-NPs 30 ppm	2.1 ± 0.02 de	0.6 ± 0.02 a	2.6 ± 0.02 a	2.4 ± 0.07 a
	Bio-NPs 40 ppm	2.2 ± 0.02 de	0.6 ± 0.01 b	2.6 ± 0.01 a	2.4 ± 0.06 a
Tall Utah 52–75	Mineral 100 kg	5.1 ± 0.03 a	0.5 ± 0.01 b	2.3 ± 0.02 d	2.2 ± 0.04 a
	Bio-NPs 20 ppm	3.6 ± 0.01 bc	0.4 ± 0.02 a	2.6 ± 0.02 a	2.3 ± 0.04 b
	Bio-NPs 30 ppm	2.4 ± 0.02 de	0.5 ± 0.01 a	2.7 ± 0.01 a	2.3 ± 0.07 b
	Bio-NPs 40 ppm	2.1 ± 0.02 e	0.5 ± 0.01 b	2.6 ± 0.02 a	2.2 ± 0.07 c

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

Potassium percentage: It was noticed that there were no significant differences between the two cultivars for both investigation seasons. Even though Balady recorded higher values of potassium percentage than Tall 52–75 (2.7%), data in Table 5 illustrate that the effects of fertilization treatments on celery potassium percentage were insignificant in both seasons. Although results (Table 5) show no significant impact on the interaction between celery cultivars and fertilizer treatments in both seasons, there were substantial differences between the two cultivars. Plants fertilized by nano-biofertilizer with a 30 ppm treatment recorded the highest potassium percentage (2.7%), while Balady recorded the lowest percentage (2.2%) when fertilized with mineral fertilizer in both seasons. Table 4 results reveal significant effects on the interaction between celery cultivars and fertilizer treatments in both seasons. There are substantial differences between Balady cv. and Tall 52–75 cultivar fertilized by nano-biofertilizer treatment (Bio-NPs-30 ppm), which recorded the highest calcium percentage (2.4%) and Bio-NPs-20 ppm fertilizer treatment, which recorded the lowest potassium percentage (2.2%) in the second season. This result may indicate that nano-biofertilization was sufficient to supply similar macro- and micronutrients in the soils to plant uptake.

3.5. Antioxidant Assay

The data in Table 6 demonstrate that celery extract with NH_4NO_3 nanoparticles-biofertilizer exhibited the most significant antioxidant activity and total phenolic content compared to the control. Furthermore, NH_4NO_3 nanoparticles increased antioxidant activity and total phenolic content in the extract of *Apium graveolens* compared to the control group. Reactive oxygen species (ROS) are produced when the high salt concentration frequently obstructs cellular electron transport across different subcellular compartments. Encouraging the build-up of non-enzymatic antioxidants such as phenolic compounds and ROS improved the antioxidant defense system [47,48]. In the first season, total phenols, flavonoids, DPPH, and ABTS antioxidants were evaluated for two cultivars, Local Egyptian

Balady and Tall Utah 52–75, under varying nitrogen source and concentration treatments. For the Local Egyptian Balady cultivar, the results indicate that applying Bio-NP treatments at 30 ppm resulted in the highest levels of total phenols and flavonoids, with values of 0.35 GAE/mL and 0.21 C.E./mL, respectively.

Table 6. Effect of biogenic nanoparticles (20, 30, and 40 ppm) on total phenolic, flavonoids, and antioxidant capacity of two celery plants (Balady and Tall Utah 52–75) compared to traditional nitrogen fertilizer (100 kg).

Genotype	Nitrogen Source and Concentration	First Season			
		Total Phenols (GAE/mL)	Total Flavonoids (C.E./mL)	DPPH (T.E./mL)	ABTS (T.E./mL)
Local Egyptian Balady	Mineral 100 kg	0.21 ± 0.01 c	0.17 ± 0.02 b	0.58 ± 0.07 c	4.3 ± 0.12 c
	Bio-NPs 20 ppm	0.24 ± 0.01 cd	0.17 ± 0.01 b	0.62 ± 0.05 bc	4.6 ± 0.09 b
	Bio-NPs 30 ppm	0.35 ± 0.02 a	0.21 ± 0.01 a	0.76 ± 0.07 a	5.0 ± 0.21 a
	Bio-NPs 40 ppm	0.35 ± 0.02 a	0.19 ± 0.02 ab	0.75 ± 0.04 a	5.1 ± 0.09 a
Tall Utah 52–75	Mineral 100 kg	0.17 ± 0.01 d	0.15 ± 0.01 c	0.47 ± 0.04 e	3.7 ± 0.13 d
	Bio-NPs 20 ppm	0.21 ± 0.04 c	0.16 ± 0.02 bc	0.51 ± 0.02 d	3.6 ± 0.11 d
	Bio-NPs 30 ppm	0.26 ± 0.05 b	0.18 ± 0.02 b	0.59 ± 0.02 c	4.2 ± 0.16 c
	Bio-NPs 40 ppm	0.26 ± 0.03 b	0.17 ± 0.02 b	0.57 ± 0.05 c	4.4 ± 0.11 bc
Second season					
Local Egyptian Balady	Mineral 100 kg	0.21 ± 0.03 d	0.17 ± 0.01 bc	0.56 ± 0.03 c	3.7 ± 0.18 c
	Bio-NPs 20 ppm	0.25 ± 0.02 c	0.19 ± 0.01 ab	0.54 ± 0.06 cd	3.9 ± 0.13 b
	Bio-NPs 30 ppm	0.31 ± 0.02 a	0.22 ± 0.02 a	0.73 ± 0.06 a	5.7 ± 0.21 a
	Bio-NPs 40 ppm	0.32 ± 0.02 a	0.18 ± 0.01 b	0.73 ± 0.03 a	5.3 ± 0.17 a
Tall Utah 52–75	Mineral 100 kg	0.19 ± 0.03 cd	0.15 ± 0.01 c	0.51 ± 0.07 d	3.1 ± 0.16 e
	Bio-NPs 20 ppm	0.22 ± 0.01 cd	0.17 ± 0.02 bc	0.53 ± 0.03 d	3.4 ± 0.13 d
	Bio-NPs 30 ppm	0.26 ± 0.02 bc	0.19 ± 0.01 ab	0.61 ± 0.03 b	3.9 ± 0.17 b
	Bio-NPs 40 ppm	0.28 ± 0.02 b	0.18 ± 0.01 b	0.59 ± 0.07 b	3.8 ± 0.09 bc

Different letter(s) in each column indicate significant variations between the treatments ($p \leq 0.05$).

Similarly, the Bio-NPs 30 ppm treatment exhibited the highest levels of DPPH and ABTS antioxidants, with values of 0.76 and 5.021 T.E./mL, respectively. These values were significantly higher than the mineral fertilizer treatment, which recorded values of 0.21 GAE/mL as total phenols, 0.17 C.E./mL as total flavonoids, 0.58 T.E./mL as DPPH, and 4.3 T.E./mL as ABTS. Conversely, for the Tall Utah 52–75 cultivar, the Bio-NPs treatment at 30 ppm also demonstrated the highest levels of total phenols, total flavonoids, DPPH, and ABTS antioxidants. These values were notably higher than those obtained from the mineral fertilizer treatment, which registered values of 0.17 GAE/mL as total phenols, 0.15 C.E./mL as total flavonoids, 0.47 T.E./mL as DPPH, and 3.7 T.E./mL as $ABTS^{+}$. Overall, the results suggest that the Bio-NPs treatment at 30 ppm consistently led to increased levels of antioxidants in both cultivars compared to the mineral treatment, indicating its potential to enhance antioxidant activity in plants.

In the second season, under different nitrogen source and concentration treatments, a similar trend was observed in the total phenols, total flavonoids, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) antioxidants for the Local Egyptian Balady and Tall Utah 52–75 cultivars. For the Local Egyptian Balady cultivar, the Bio-NPs treatment at 30 ppm exhibited the highest levels of total phenols, total flavonoids, DPPH, and ABTS antioxidants, with values of 0.31, 0.22, 0.73, and 5.7, respectively. These values were significantly higher compared to the mineral treatment, which recorded values of 0.21 GAE/mL (total phenols), 0.17 C.E./mL (total flavonoids), 0.56 T.E./mL (DPPH), and 3.7 T.E./mL (ABTS). Similarly, for the Tall Utah 52–75 cultivar, the Bio-NPs treatment at 30 ppm demonstrated the

highest levels of total phenols, total flavonoids, DPPH, and ABTS antioxidants. These values were notably higher than those of the mineral treatment. Overall, the results reaffirm the efficacy of the Bio-NPs 30 ppm treatment in enhancing antioxidant activity in both cultivars compared to the mineral treatment, highlighting its potential role in promoting plant health and stress tolerance.

The increase in vegetable production is due to nanoparticles affecting plants by many physiological and morphological changes. Depending on the nanoparticle's properties, they have important properties, such as small size, different shapes, and larger area-to-weight ratio. Bio-fertilization in the form of nano compounds improved the growth of plants, as well as increased their active substances. These are among the most essential compounds to maintain human health [49].

4. Discussion

The current study aims to find the most suitable fertilizer to enhance growth and yield parameters, phenolic and flavonoid accumulation, and decrease nitrogen content in celery plants. However, excessive chemical fertilizers of chemical origin increase the risk of environmental pollution and lead to a high concentration of nitrates in the consumed parts of vegetables. Nitrogen is a significant nutrient and a building block of proteins, making many biochemical reactions on which life is based possible. Nitrogen deficiency most often results in stunted growth, slow growth, and chlorosis, and the underside of leaves appear purple from an accumulation of anthocyanin pigments [50].

Nanotechnology is one of the most essential tools in modern science, yet only a few attempts were made to apply these advances to increase crop productivity [51]. It is possible to develop microorganisms as bio-nanofactories to synthesize agriculturally essential particles [52]. Although it is known that microorganisms, such as bacteria, yeast, and now fungi, play a vital role in the remediation of toxic metals by reducing the metal ions, this was considered attractive as nanofactories very recently [53]. Fungi are a relatively recent addition to the list of microorganisms used to synthesize nanoparticles [54]. Using fungi is potentially exciting since they secrete large amounts of enzymes and are simpler to manage in the laboratory. In the biosynthesis of metal nanoparticles by a fungus, extracellularly secreted enzymes are produced, which reduce the metal salt of a macro or micro scale into a nanoscale diameter through the catalytic effect. The negative electrokinetic potential of microorganisms enables them to attract the cations and triggers the biosynthesis of metal and metal oxide nanoparticles [55,56].

The current results indicate that the growth indicators of celery and dill plants were significantly affected by adding bio-fertilizer treatments in both seasons. In most cases, applying the 30 and 40 ppm Bio-NPs treatment increased plant height and wet and dry weight considerably. In the current study, bio-fertilization improved the plant height compared to the fertilized plants by mineral nitrogen [57,58]. The most minor growth occurred with the treatment (mineral 100 kg) alone. At the same time, using 100 kg mineral treatment led to increased total chlorophyll and carotenoids in two plots in both seasons. The positive effect on growth traits, total chlorophyll, and total carotenoids in response to biofertilizers may be attributed to increased moderation in plant tissues [59]. Biofertilizers produced by microorganisms such as bacteria may also increase the synthesis of endogenous plant hormones, such as Indole-3-acetic acid (IAA), gibberellins (Gas), and Cytokinins (CKs), which play a vital role in the formation of an extensive active root system, allowing more nutrients and their uptake from the soil, and finally, accelerating plant growth. The previous results agree to some extent with the results [60].

The preference for treated plants is due to the ease and speed of movement of bio-fertilizer particles in the form of nanoparticles, which helps the plant perform all vital processes well [61–65]. It increases vitamin C content in plant leaves using biofertilizer treatments (Bio-NPs). This may be due to increased occupancy of the plant's root zone due to adding fertilization treatments, which is reflected in the plant's absorption of nutrients and confirms that the precedent of vegetative growth as similar in fennel plants [66].

Macro-nutrients N, P, K, and Ca content varied in fertilized celery plants regardless of the fertilizer source (Table 5). Coarse mint's P and N content increased with arbuscular mycorrhizal fungi inoculation [57]. Organic fertilizers improved fennel growth due to their effect on the availability of most micro- and macro-nutrients compared to chemical fertilizers [67]. Organic additives (farm manure and chicken manure) and mineral NPK (50 and 100% of RD) led to the highest N, P, and K content in fennel plants [68]. Previous studies demonstrated the accumulation of N, P, and K in plants in response to chemical, organic, and biofertilizers, as reported in caraway [69], chamomile [70,71], cabbage [72], kale [70], and coriander [73]. Phenols and flavonoids are bioactive components in celery leaves that act as antioxidants and have various preventive and therapeutic effects. Data from Table 5 show that using biofertilizers in nanoparticles positively enhanced TPC and TFC compared to celery fertilized with mineral nitrogen in both seasons. Indeed, these fertility treatments resulted in increased photosynthesis rates and significantly improved element accumulation, which catalyze the biosynthesis of carbon-based secondary metabolites, such as phenolic compounds and flavonoids [74].

Combining nanotechnology with biofertilizers strengthens plant growth and mitigates environmental stress. These techniques are more economically and environmentally sustainable, highly versatile, and are long-lasting farming tools [75]. Despite the fact that this study provides valuable insight to turn into more applications of bio nano-fertilizers in vegetable cultivation, comprehensive research and understanding of the long-term impacts and potential risks associated with the use of bio-nano-fertilizers in agriculture and determining the most appropriate concentration for individual crops are still lacking.

5. Conclusions

This study highlights the effectiveness of nano-biofertilization with nitrogen (Bio-NPs-30 ppm), significantly enhancing plant growth, fresh and dry weight, and nutrient content. Using nano-biofertilizers accelerates plant nutrient absorption, improving growth rates and nutritional value. Additionally, it boosts antioxidant levels in celery crops, enhancing their overall health benefits. Moreover, nano-biofertilizers reduce nitrogen accumulation in plant leaves, promoting plant growth. These findings emphasize the importance of applying bio-based nanomaterials, such as Bio-NPs, as sustainable alternatives to traditional mineral fertilizers. Bio-NPs offer a holistic approach to improving celery production while mitigating environmental ecotoxicity by minimizing nitrogen content and enhancing growth and antioxidant activity. A comparative analysis may be recommended for future research to compare the effectiveness and safety of various types of bio-nano-fertilizers with varying compositions and concentrations to identify the most efficient formulations for building a resilient agroecosystem.

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