

Effect of *Scenedesmus obliquus* extract and its biosynthesized zinc oxide nanoparticles as foliar application on growth and yield of tomato grown in late summer seasons

Abuo El-Kasem, S.A.A.^{1*}, Eman S.E. Tony¹, O.M. Darwesh² and I.A. Matter²

¹ Vegetable Research Department, Horticulture Research Institute, Agriculture Research Center, 12619 Giza, Egypt.

² Agricultural Microbiology Department, National Research Centre, 33 EL-Buhouth St., Dokki, 12622 Cairo, Egypt.

Abstract

This study focused on the use and application of microbial Nano- biotechnology, specifically its impact on tomato plant traits and soil microbial quality. In this regard, the microalga *Scenedesmus obliquus* was utilized for the green biosynthesis of zinc nanoparticles to be applied along with microalgal cell extract as a foliar fertilizer to enhance the growth and productivity of tomatoes. Biosynthesized ZnO-NPs at concentrations of 150, 200, and 250 ppm were used as foliar spray in batches with or without microalgal extract (3 cm/L) on tomato plants. For comparison, chemically synthesized ZnO-NPs at the same concentrations were used. Results showed that all interaction treatments showed statistically similar or higher values for each trait compared to the corresponding control groups (control without algae and without NPs). The treatment with algal extract combined with 250 ppm of bio-NPs demonstrated the most significant increases: 37.0% increase in total fruit yield, 43.1% increase in marketable yield, 369.4% increase in total microbial activity, 298.8% increase in dehydrogenase activity in soil, and increases of 74.8%, 182.7%, and 104.2% in zinc accumulation in root, leaves, and fruit, respectively, compared to the control treatments. These results suggest that phyco-synthesized ZnO NPs may stimulate activity in meristematic cells, potentially by activating essential biochemical pathways, leading to enhanced biomass accumulation in tomato plants.

Keywords: Microalgal extract; Nanoparticles Biosynthesis; *Scenedesmus obliquus*; Foliar fertilization; Tomato (*Solanum lycopersicum L.*).

1. Introduction

Tomato (*Solanum lycopersicum L.*) is one of the most important and popular vegetables cultivated for the consumption of their edible fruits, which are included in many diets and foods around the world. It is an important source of vitamin C, vitamin K, beta-carotene, lycopene, folate, potassium, and phenolic compounds. Calories: 22.5, fat: 0.25 (g), sodium: 6.25 (mg), carbohydrates: 4.86g. fiber: 1.5g, added sugars: 0

g, protein: 1.1g., Recent research highlights the benefits of consuming fresh tomatoes for maintaining human health. These benefits include supporting the immune system, fighting viruses, and control blood pressure. Tomatoes may also have anti-cancer properties, reduces the risk of heart disease and cholesterol, prevents constipation, reduces the risk of type 2 diabetes, improves insulin levels during fasting, and reduces the possibility of Alzheimer's disease (Tsitsimpikou *et al.*, 2014; Rowles *et al.*, 2018; Senkus *et al.*, 2019; Ali *et al.*, 2020; Collins *et al.*, 2022; Leh *et al.*, 2022; Rock *et al.*, 2020; Sass and Tolentino, 2024). Tomato is classified as the second most important crop globally, after

*Corresponding author: Sameh A.A. Abuo El-Kasem

Email: samehaoelkaseem7@gmail.com

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potatoes and Egypt ranks fifth after China, India Turkey, and the USA, respectively, in the world's tomato production (Abdelkader *et al.*, 2022). The total production of Tomato in Egypt is about 6,275,443 tons/year with a total cultivation area of 143,618 Hectares (ha) with an average production of 41.6 tons/ha. In regarding, local production in Egypt, Tomato production ranks first among vegetable crops, and fifth in production after sugar cane, sugar beet, wheat, and corn (FAO, 2022). Fertilizer application is a cornerstone for achieving optimal tomato yield and quality, as is the case for many other vegetables and crops. However, the effectiveness of fertilizer application on tomatoes is influenced by a multitude of environmental and soil-related factors. These factors can significantly decrease nutrient bioavailability or plant uptake. Such limitations are particularly pronounced in newly reclaimed areas like North Sinai, Egypt, posing a significant challenge to the sustainability of agricultural production in the region (Abuo El-Kasem and Mahmoud, 2017). These challenges can be addressed through modern and safe agricultural practices, including the use of foliar fertilization with natural growth stimulants such as microalga extracts. This natural, environmentally friendly and cost-effective foliar fertilizer stimulates the plant's absorption of nutrients, and increases the plants' ability to resist adverse surrounding conditions, thus increasing growth and productivity (Abo-Sedera *et al.*, 2015). Numerous macronutrients, microelements, vitamins, cytokinins, auxins, amino acids, and abscisic acid (ABA) are abundant in microalgal extracts. These natural compounds act as natural growth regulators in plants, which when used as foliar fertilizers at the right ratio and timing lead to improved growth and increased crop productivity (Durand *et al.*, 2003; Ordog *et al.*, 2004). The composition and effectiveness of algal extracts as plant bio-stimulants varies depending on the species of microalga used, their growth conditions, and the method of extraction (Matysiak *et al.*, 2010;

Noha *et al.*, 2018). On the other hand, zinc plays a pivotal role in supporting essential functions in plants, such as photosynthesis, enzyme activation, hormone synthesis, and chlorophyll formation. Enzymes, as biological catalysts, rely on zinc for proper activation in various metabolic pathways, including those for nutrient uptake, energy production, and defense mechanisms. Crucial for the development of plant roots, zinc aids in efficient nutrient absorption and enhances the plant's ability to withstand environmental stresses. Additionally, zinc is integral to chlorophyll formation, essential for converting sunlight into chemical energy during photosynthesis. Insufficient zinc levels can disrupt enzyme function, impair chlorophyll synthesis, and hinder overall plant development. Therefore, maintaining optimal zinc levels in the soil and ensuring proper uptake by plants are vital for their health, growth, and productivity (Cakmak, 2000; Broadley *et al.*, 2012; Layam *et al.*, 2016). Zinc deficiency in food crops is widespread, with nearly 50% of productive agricultural soils being deficient in zinc (Sillanpaa, 1990), and approximately 50% of the world's human population also suffering from zinc deficiency (Hotz and Brown, 2004). Zinc treatment is therefore crucial for agricultural yield as it improves plant vigor and increases stress tolerance in possibly zinc-deficient soils. Biosynthesized Zinc nanoparticles (Zn-NPs) is a reduced form of the mineral zinc, which is manufactured by reducing zinc using biological reducing agents found in either plant or microbial extracts. Zn-NPs has many important applications in various medical, industrial, environmental and agricultural fields. One of these agricultural applications is its use as a foliar fertilizer to treat zinc deficiency in plants, to stimulate growth, and to combat some physiological and microbial diseases (Al Jabri *et al.*, 2022). *Scenedesmus* sp., a fresh water microalga, offers a wide range of biotechnological applications due to the activity of its living cells and the presence of bioactive

compounds in its extracts (Chacón-Lee, and Gonzalez-Marino, 2010; Guedes *et al.*, 2012). The aqueous extract of this microalga has been proven to have a stimulating effect on plant growth, when used as a foliar fertilizer for many plants, including tomatoes (Supraja *et al.*, 2020). In addition, *Scenedesmus* extract was applied as reducing agent for nanoparticles biosynthesis e.g. Zn-NPs and AgNPs (Darwesh *et al.*, 2019; Saleh *et al.*, 2022). Algae-mediated synthesis of nanoparticles offers several advantages, primarily in reducing the toxicity associated with traditional chemical methods of nanoparticle production (Ogunyemi *et al.*, 2019). The unique combination of physical, chemical and biological properties makes zinc oxide nanoparticles (ZnO-NPs) stand out among metal oxide nanomaterials. The ZnO formula has a wide radiation absorption spectrum, a high electrochemical coupling coefficient, strong photostability, and high chemical stability (Alavi *et al.*, 2021).

Therefore, the current study aims to evaluate the effect of *Scenedesmus* algal extract and/or its biosynthesized ZnO-NPs as an environmentally friendly foliar fertilizer on growth and productivity of tomato plants compared to the chemically prepared ZnO-NPs.

2. Materials and methods

Two experiments were carried out in the consecutive seasons of 2022 and 2023 at the Agricultural Research Station in El-Arish, North Sinai Governorate, Egypt. The aim was to study of tomato plants response to the microalga *Scenedesmus obliquus* used in the green synthesis of zinc nanoparticles. These nanoparticles were then applied along with microalga cell extract as foliar fertilizer to enhance tomato growth and productivity. Biosynthesized ZnO-NPs at concentrations of 150, 200, and 250 ppm were used as foliar spray in batches with or without microalga extract (3 cm/L) on tomato plants. For comparison, chemically synthesized Zn-NPs at

the same concentrations were employed in a split-plot design with three replicates. Normal agricultural practices were implemented, consistent with recommendations from the Egyptian Ministry of Agriculture and Land Reclamation for commercial tomato production, including the use of a drip irrigation system four times a week. The treatments included spraying with the algal extract, with two treatments: spraying with the extract and no spraying (control), serving as the main plot treatment.

In the sub-plot treatment, zinc was applied in nano form under seven treatments, with the first being a control (without zinc), and the other six treatments divided into two procedures for the synthesis of ZnO-NPs (biosynthesis and chemical synthesis of ZnO-NPs), each including three concentrations: 150, 200, and 250 ppm.

2.1. Microalga and cultivation conditions

The freshwater microalga *S. obliquus* was provided from the “Department of Agricultural Microbiology, National Research Centre”. The microalga had been previously isolated and characterized as *S. obliquus* NRCIbr1 accession No.: KY621475 (Eida *et al.*, 2018). Bold Basal Medium (BBM) was utilized for maintaining and culturing the microalga. BBM, as described by Barsanti and Gualtieri (2006), comprises the following components per liter: KH_2PO_4 , 175 mg; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 25 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 75 mg; NaNO_3 , 250 mg; K_2HPO_4 , 75 mg; NaCl , 25 mg; H_3BO_3 , 11.42 mg and trace elements including $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 8.82 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.44 mg; MoO_3 , 0.71 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.57 mg; $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.49 mg; Na_2EDTA , 50 mg; KOH , 3.1 mg; FeSO_4 , 4.98 mg and 1 μL H_2SO_4 (Conc.). Cultivation was carried out in 5-Litter air-bubbled glass flasks under continuous lighting with white, fluorescent lights. The dry weight of the samples was determined during the culture process by filtering them through 0.45 μm glass fiber filters and drying them at 105°C to monitor biomass content. The cultivation process was stopped when the concentration reached 2.0

g L⁻¹. The biomass was then harvested by centrifugation for 10 minutes at 6000 rpm, twice washed with distilled water, suspended in distilled water at 20 g L⁻¹, and frozen at -18 °C until needed. Aqueous cell extract (post-disruption) was employed for foliar fertilizer application, while the cell-free supernatant was used for ZnO-NPs biosynthesis.

2.2. Microalga disruption and extraction

The aqueous extract of *S. obliquus* microalga was prepared following the method outlined by Di Caprio *et al.* (2022) and Saleh *et al.* (2022), with minor adjustments. Briefly, concentrated fresh cells of the microalga underwent three sequential freezing and thawing cycles to disrupt the cells, followed by 20 minutes of sonication using a probe-sonicator. The resulting mixture of disrupted cells was then diluted tenfold with distilled water, restoring the final biomass concentration to 2 g/L, as it was prior to harvesting.

2.3. Synthesis of ZnO-NPs

2.3.1. Biosynthesis

The biosynthesis approach of ZnO-NPs using cell-free culture filtrate of microalga *S. obliquus* was used as reported by Saleh *et al.* (2022) with some minor modifications. Briefly, the microalgal cell free supernatant was incubated overnight (in dark) with an equal volume of 0.5% zinc acetate solution at 100 rpm at 28°C. Centrifugation at 10,000 rpm for 15 minutes was used to gather the precipitates. Zn-NPs formation was initially confirmed using sediment scanning spectrophotometry at 350 nm (Sidhu *et al.*, 2022). The precipitated particles were then cleaned three times with deionized water and then twice with 100% ethanol before being dried at 50°C in an oven until a constant weight were obtained. After being dried and milled to the ideal consistency, the Zn-NPs were gathered and further examined.

2.3.2. Chemical synthesis

Chemical reduction (bottom-up approach) was employed in this investigation to transform Zn

metal ions into their Nano forms in accordance with accepted practices in the literature (Aboud *et al.*, 2020). To put it briefly, 2% zinc sulfate was mixed vigorously and continuously with 1% sodium hydroxide solution (as a reducing agent) until white precipitates were visible. The precipitates were gathered, cleaned, and dried in the same manner as previously mentioned for the Zn-NPs that were biosynthesized.

2.3.3. Characterization of ZnO-NPs

The produced bio- and chemical-synthesized ZnO-NPs were analyzed at the National Research Centre, Egypt, using high-resolution transmission electron microscopy (JEOL 2100, Japan) to determine their size and shape (Sidhu *et al.*, 2022). Fourier Transforms Infrared spectroscopy (FTIR) was conducted to identify phytochemicals potentially involved in capping, reduction, and effective stabilization of the synthesized ZnO-NPs. The materials were scanned using an FTIR spectrometer (Agilent system Cary 630 FTIR model, USA) with infrared light in the range of 4000 to 400 cm⁻¹. The resulting spectrum data were compared with reference charts to determine the presence of functional groups in the sample(s) (El-Shanshoury *et al.*, 2020). Additionally, an X-ray diffractometer (XRD-6000 series by Shimadzu equipment) was utilized to analyze the crystalline structure of the Zn-NPs, as noted by Djearmane *et al.* (2019). Surface morphology and high-resolution photographs of the samples were revealed using a scanning electron microscope (SEM). Additionally, an energy-dispersive X-ray analyzer was used to identify elements and provide quantitative compositional data.

The physical and chemical analyses of the experimental soil in average of the two seasons: Physical properties include organic matter content (0.16%) and textural class (sandy loam). Chemical properties include pH (7.6), electrical conductivity (EC) (2.12dS/m), calcium carbonate (CaCO₃) content (13%), and available nutrients in parts per million (ppm), categorized as follows: Macro-elements: nitrogen (N) (15 ppm),

phosphorus (P) (13 ppm), and potassium (K) (281 ppm); Micro-elements: zinc (Zn) (0.14 ppm), manganese (Mn) (1.03 ppm), iron (Fe) (0.9 ppm), and copper (Cu) (0.39 ppm).

2.4. Tomato seedlings and cultivation conditions

Seedlings of tomato (*Solanum Lycopersicon L.*) cv. Lojain F₁, produced by Enza Zaden, the Netherlands, were obtained from a commercial nursery in El-Arish, Egypt, at the age of 35 days. Transplanting was carried out on the 1st of May in two consecutive seasons. The plot area was 25.2 m², with three dripper lines, each measuring 6 m in length and 1.4 m in width. Each plant was placed on a dripper line, with a spacing of 0.5 m between each plant on the same line.

In terms of algal foliar spraying, all experimental tomato plants were sprayed from the bottom to the top, adhering to the commonly recommended protocol as a fine mist until runoff, ensuring comprehensive coverage of all plant organs. 3 cm/L algal extract were administered four times, with the initial foliar spraying commencing 30 days after the seedlings were transplanted, at 20-day intervals throughout the plants' growing season.

Regarding the foliar application of Nano ZnO treatments, tomato plants began receiving the treatments 40 days after transplanting. The nanoparticles were dispersed in deionized water using a magnetic stirrer (ANZESER SH-2) for 30 minutes. The resulting aqueous solutions were then directly sprayed onto the leaves using an atomizer. Spraying alternated between nanoparticle Zn spray and the algae extract spraying treatment with an interval of ten days, ensuring that the spraying cycle for both the algae extract spraying and Nano-zinc treatments were completed every 20 days. The control plants treatment were not sprayed.

2.5. Experimental parameters

2.5.1. Vegetative growth

A random sample of 5 plants from each plot was taken after 65 days from transplanting and the

following data were recorded: Plant height, No. of branches and root fresh weight as well as the dry weight of organs plants *i.e.*, root, leaves and branches.

2.5.2. Estimation of chlorophyll content in the tomato plant leaves

The chlorophyll contents of tomato leaves was measured by grounding 0.2 g fresh leaf with 5 mL of 80% acetone (for extraction) and centrifuged at 5000 rpm for 10 min. Subsequently, the absorbance of extracted supernatant was measured at 645 and 663 nm after adjusting its volume up to 10 mL with 80% acetone (Perveen *et al.*, 2010). The following equations were used to calculate chlorophyll a (Chl a) and chlorophyll b (Chl b) contents:

$$\text{Chl a (mg/g)} = [12.7 (\text{OD}_{663}) - 2.69 (\text{OD}_{645})] \times V/1000 \times w.$$

$$\text{Chl b (mg/g)} = [22.9 (\text{OD}_{645}) - 4.68 (\text{OD}_{663})] \times V/1000 \times w.$$

Where: OD₆₄₅ = Absorbance at a wavelength of 645 nm. OD₆₆₃ = Absorbance at a wavelength of 663 nm. V = Final volume; W = weight of leaf tissue.

2.5.3. Zinc content:

Upon conclusion of the experiment, the fruits, leaves, and roots were separated. The roots were initially washed with ultrapure water low in organics, followed by rinsing with 5 mM CaCl₂ at 4°C for 15 minutes, and then with water again (Barabasz *et al.*, 2012). Subsequently, the collected plant parts were dried in an oven at 55°C for 4 days until a constant biomass was achieved, after which they were utilized to determine the concentration of zinc. The zinc concentration in the plant components (fruits, leaves, and roots) was assessed using Inductively Coupled Plasma–Mass Spectrometry (ICP MS). The digestion and extraction processes were conducted using a closed-vessel microwave system (Ethos-1600, Milestone, Sorisole, Italy) equipped with a fiber-optic temperature sensor. Each sample item (50 mg) was placed in a 50 mL glass vessel along with 7 mL of diluted nitric acid solution (65%) and 1 mL of H₂O₂ (30% v/v). Two

stages of heating were performed for ten minutes each at 200°C (Alsabhan *et al.*, 2022). The digested samples were subsequently analyzed using ICP-MS analysis at the National Research Centre in Egypt, with an external calibration performed using a 10-ppm concentration multi-element standard.

2.5.4. Fruit and yield

At the time of harvest, ten plants were randomly selected from each plot to assess fruit and yield characteristics, including average fruit weight (grades A and B), as well as total fruit yield (tons per fed) and marketable fruit yield (tons per fed).

2.6. Soil's microbial enzyme activities:

2.6.1. Total microbial enzyme activities (TMA)

Based on the rate of fluorescein diacetate (FDA) hydrolytic activity, the total microbial enzyme activities of soils were determined using a modified version of the Patle *et al.* (2018) approach. To put it briefly: 50 milliliter capped centrifuge tubes were filled with two grams of rhizosphere soil samples (in triplicate). The reaction was started with the addition of 0.2 mL of 0.1% FDA (in acetone) and 15 mL of potassium phosphate buffer (60 mM, pH 7.6). In a rotating shaker, tubes were incubated horizontally at 30°C for 20 minutes. Following incubation and color development, 15 mL of chloroform/methanol (2:1) was added, and the reaction was halted by vortexing for one minute. The tubes were centrifuged at 5000 rpm for 10 minutes in order to separate the chloroform layer and spin down the soil and turbidity. Using spectrophotometric measurement at 490 nm, the produced colored fluorescein in the chloroform layer was compared to fluorescein standards. An expression for the total amount of soil microbial activity was FDA hydrolysis values (μg of released fluorescein g⁻¹ soil).

2.6.2. Dehydrogenase activity (DHA)

With a few adjustments, the dehydrogenase activity in soil samples was measured using the

procedure outlined by Navnage *et al.* (2018). In a nutshell, 0.2 ml of a 3% 2,3,5 triphenyltetrazolium chloride (TTC) solution is applied to one gram of soil in a 15 ml screw-capped tube. 0.5 ml of a 1% glucose solution was added to each tube, sealed with plastic stoppers, and incubated for 24 hours at 28 °C. Ten milliliters of methanol were used to extract the red-colored triphenyl tetrazolium formazan (TPF) that had developed after the sample was incubated, shaken for one minute, and left to stand in the dark for six hours. Each sample's supernatant is then filtered into a 50 ml conical flask, and the filtrates are quantified using a spectrophotometer at 485 nm.

2.7. Statistical analysis

All data were statistically analyzed applying analysis of variance (ANOVA) according to Gomez and Gomez (1984) through ASSISTAT Version 7.7 en (2017) - Website <http://www.assistat.com> By Francisco de A. S. e Silva, UFCG-Brazil (Silva and Azevedo 2016). The means of chosen parameters were compared employing the LSD test at the significance level of $p=0.05$.

3. Results

3.1. ZnO-NPs characterization

The present study investigated the impact of ZnONPs on plant growth, chlorophyll content, zinc concentration in plant organs, and fruit yield traits. Nanoparticles suspended in deionized water were utilized to observe the morphology and dimensions of ZnONPs through TEM. The resulting images predominantly depicted individual primary particles and aggregated ZnO NPs, primarily spherical in shape, with sizes ranging from 4 to 27 nm (Fig. 1).

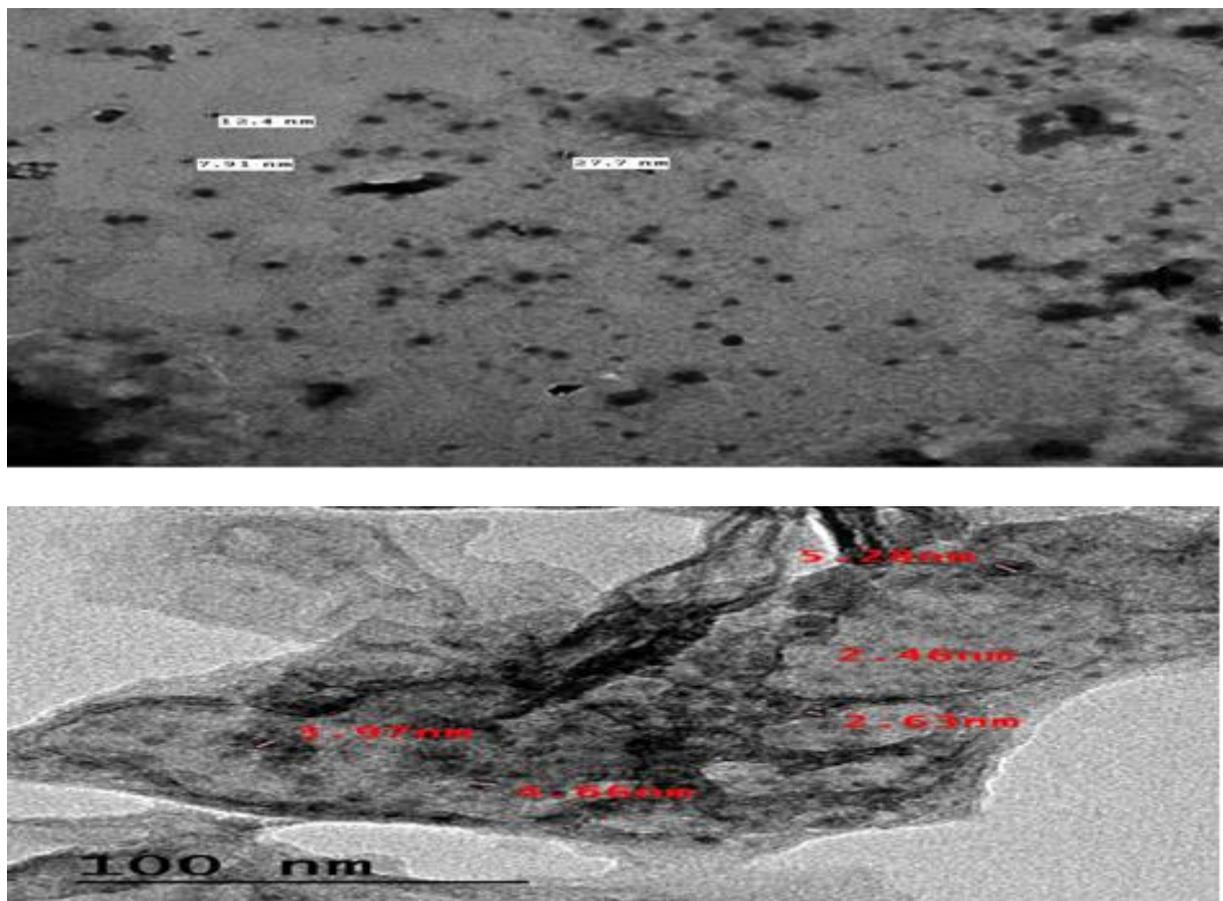


Figure 1. Individual primary ZnO nanoparticles and aggregates were observed under the transmission electron microscope.

3.2. Vegetative growth parameters:

3.2.1. Effect of algal spraying:

The results illustrated in Table 1 indicate that the algal spraying positively affected the studied vegetative growth characteristics (plant height, No. of branches, root fresh weight, and the dry

weight of root, branches, and leaves as well as chlorophyll a and b) comparing with the control (non-spray treatment) during the study seasons. Algal spraying treatment significantly surpassed the control in all abovementioned traits except chl-a in both seasons.

Table 1. Vegetative growth parameters, and chlorophyll content of tomato plants as affected by alga spraying at 2022 and 2023 seasons

Characters	Treatments	Non-spray		*Algal spray		LSD 0.05 %	
		2022	2023	2022	2023	2022	2023
	RFW	70.64	78.09	76.82	82.19	1.451	1.189
	RDW	12.19	13.60	13.72	14.91	0.911	0.883
	PH	93.03	96.08	97.11	99.05	2.730	1.492
	NB	7.48	8.05	8.00	8.41	0.446	0.178
	BDW	50.25	54.12	57.32	60.01	1.843	1.661
	LDW	98.73	101.37	110.94	114.17	1.874	2.228
	Chl-a	3.29	3.61	3.43	3.75	ns	ns
	Chl-b	2.77	2.88	2.93	3.11	0.144	0.158

*Aqueous extract of *S. obliquus* microalga RFW: Root fresh weight, RDW: Root dry weight, PH: Plant height, NB: number of branches, BDW: Branches dry weight, LDW: Leaves dry weight, Chl-a and b: chlorophyll a and b.

3.2.2. Effect of Nanoparticles (NPs)

Data presented in Table 2 and Fig.2a, and 2b show the effect of foliar applications, i.e., the two synthesis procedures of nanoparticles in three different concentrations each on vegetative growth parameters and photosynthetic pigment of tomato.

It is clearly illustrated that all foliar applications significantly increased PH(plant height), RFW (root fresh weight), NB (number of branches) BDW (branches dry weight) LDW (leaves dry weight)), RDW (root dry weight), and compared with the control (Fig. 2a) except, 150 and 200 ppm concentrations of Met-ZnONPs (Metallic Zinc Oxidase Nanoparticles) which both slightly increased RDW in both seasons, and LDW in 1st season as well as 150 ppm concentration of Met-ZnONPs effects on NB and LDW in 1st and 2nd seasons, respectively. However, it was noticed that the high concentrations (250 ppm) of the two synthesis procedures of nanoparticles solutions gave a significantly highest enhancement than, or equal to, other concentrations (100 and 200 ppm) of the same ZnONPs procedure in all vegetative

traits with no significant differences between the highest concentration of Met-NPs (250 ppm) and the lowest one of bio-NPs (150 ppm) in all vegetative traits in both seasons except LDW and NB in 1st and 2nd season, respectively in which 150 ppm bio-NPs exhibited the significantly highest increased.

Spraying metallic and bio-nanoparticles increased all vegetative growth and chlorophyll traits such as BDW, PH, LDW, RDW, RFW, NB, Chlorophyll a (Chl-a), and Chlorophyll b (Chl-b) by up to 45.50%, 23.24%, 77.81%, 59.52%, 38.22%, 40.27% (Fig 2a), 33.60%, and 51.57% (Fig. 2b), respectively on average in both seasons. The concentration of 250 ppm Bio-NPs exhibited the highest increment compared to the control treatment. However, there were no significant differences between the three concentrations within the Met-NPs in RDW, NB, and BDW traits and within Bio-NPs in RDW (Fig.2a), Chl-a, and Chl-b (Fig.2b). The minimum values of vegetative growth traits were observed in untreated plants

Table 2. Vegetative characteristics of tomato plants as affected by Metallic (Met-NPs) or biological (Bio-NPs) ZnO-nanoparticles foliar spray (combined algal sprayed and non-sprayed treatments)

Characters	Treatments	Control	Met-NPs			Bio-NPs			LSD 0.05 %
		WZn	150 ppm	200 ppm	250 ppm	150 ppm	200 ppm	250 ppm	
RFW	2022	60.41	67.06	69.89	74.27	77.28	81.15	86.04	3.96
	2023	67.46	78.33	77.55	79.68	82.1	85.2	90.7	2.973
RDW	2022	9.38	10.94	12.01	12.72	13.88	15.28	16.48	2.691
	2023	11.64	12.84	13.36	13.98	15.03	15.91	17.05	2.208
BDW	2022	77.39	88.91	97.69	101.21	106.97	123.32	138.34	5.256
	2023	79.56	92.1	100.14	106.94	109.48	125.43	140.74	4.153
LDW	2022	44.34	49.68	51.24	52.55	55.7	56.66	66.35	5.57
	2023	48.41	52.75	54.36	57.07	58.31	59.94	68.6	6.153
PH	2022	84.88	91.53	93.85	97.23	97.01	97.69	103.3	6.33
	2023	86.52	93.79	95.71	98.63	99.06	101.32	107.93	3.73
NB	2022	6.33	7.00	7.33	7.67	8.00	8.5	9.33	0.94
	2023	7.17	7.67	7.83	8.00	8.5	8.83	9.6	0.445
Chl-A	2022	2.77	2.90	3.12	3.32	3.69	3.84	3.89	0.13
	2023	3.19	3.42	3.59	3.83	3.78	3.89	4.07	0.23
Chl-B	2022	2.13	2.45	2.75	3.00	3.15	3.21	3.26	0.32
	2023	2.35	2.64	2.85	3.09	3.19	3.30	3.53	0.29

Control : without zinc application; RFW: Root fresh weight, RDW: Root dry weight, PH: Plant height, NB: number of branches, BDW: Branches dry weight, LDW: Leaves dry weight, Chl-a and b: chlorophyll a, and b

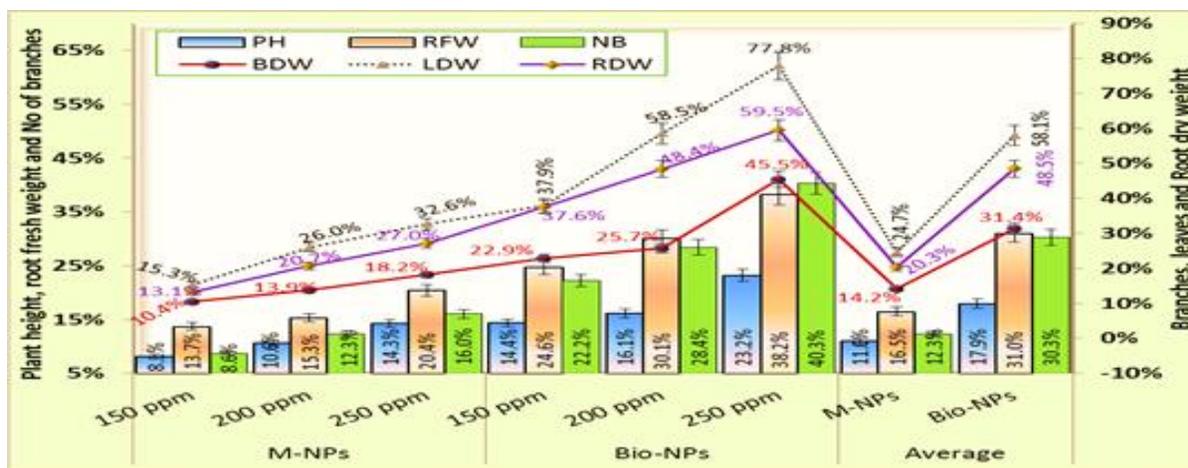


Figure 2: (a). shows a percentage increase in plant growth characteristics on average of both seasons as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles compared to the control for the following parameters: PH (plant height), RFW (root fresh weight), NB (number of branches), BDW (branch dry weight), LDW (leaf dry weight), and RDW (root dry weight)

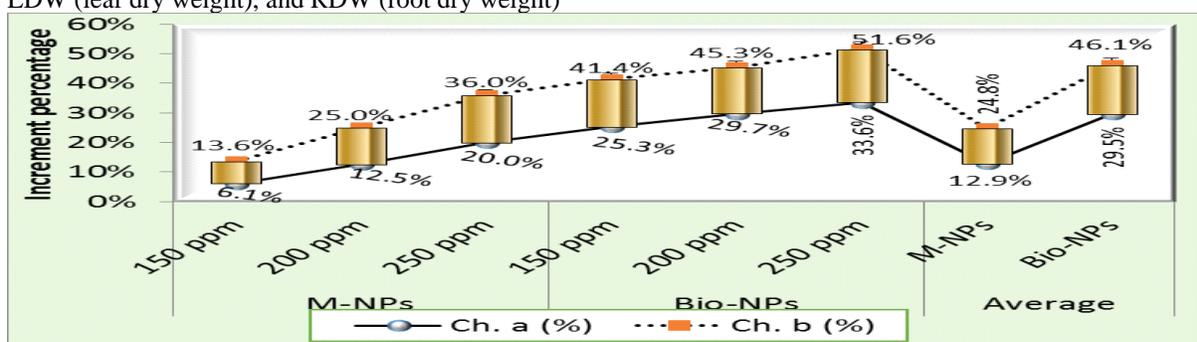


Figure 2: (b). shows a percentage increment in chlorophyll characteristics of tomato plants as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray (combined of algal sprayed and non-sprayed treatments).

3.2.3. Interaction effect

The interaction effects between algal spraying and ZnO-NPs foliar spray are depicted in Fig.3a&b showcasing their impact on vegetative growth and chlorophyll traits. The Fig. 3 illustrates the studied traits of tomato plants treated with two bio spray methods (control without algae and algae extract spray) in conjunction with various ZnO-NPs treatments. It is evident that all combined treatments resulted in statistically equivalent or increased values across all vegetative growth (Fig 3a) and chlorophyll traits (Fig3 b) compared to the respective control treatments (control without algae × control without NPs), highlighting the beneficial role of the algal extract.

Upon treating plants with the algal extract (AlgSp) in combination with 250 ppm Bio-NPs, the highest values for all vegetative growth traits were observed in Figure 4. This treatment resulted an increment percentage (as average of both seasons) by 88.5%, 67.8%, 65.6%, 64.8%, 46.6%, 45.0%, 41.0% and 30.3% in descending order for LDW, RDW, Chl-b, BDW, NB, RFW, Chl-a and PH over the control treatments (control without alga × control without NPs), respectively (Fig. 3b&4) followed by 250 BioNPs × Non-spray and 200 ppm BioNPs × AlgSp with no significant differences between them in all vegetative growth and chlorophyll except leaves dry weight in which 250 ppm Bio-NPs without algal spraying was higher affects than 200 ppm BioNPs x AlgSp.

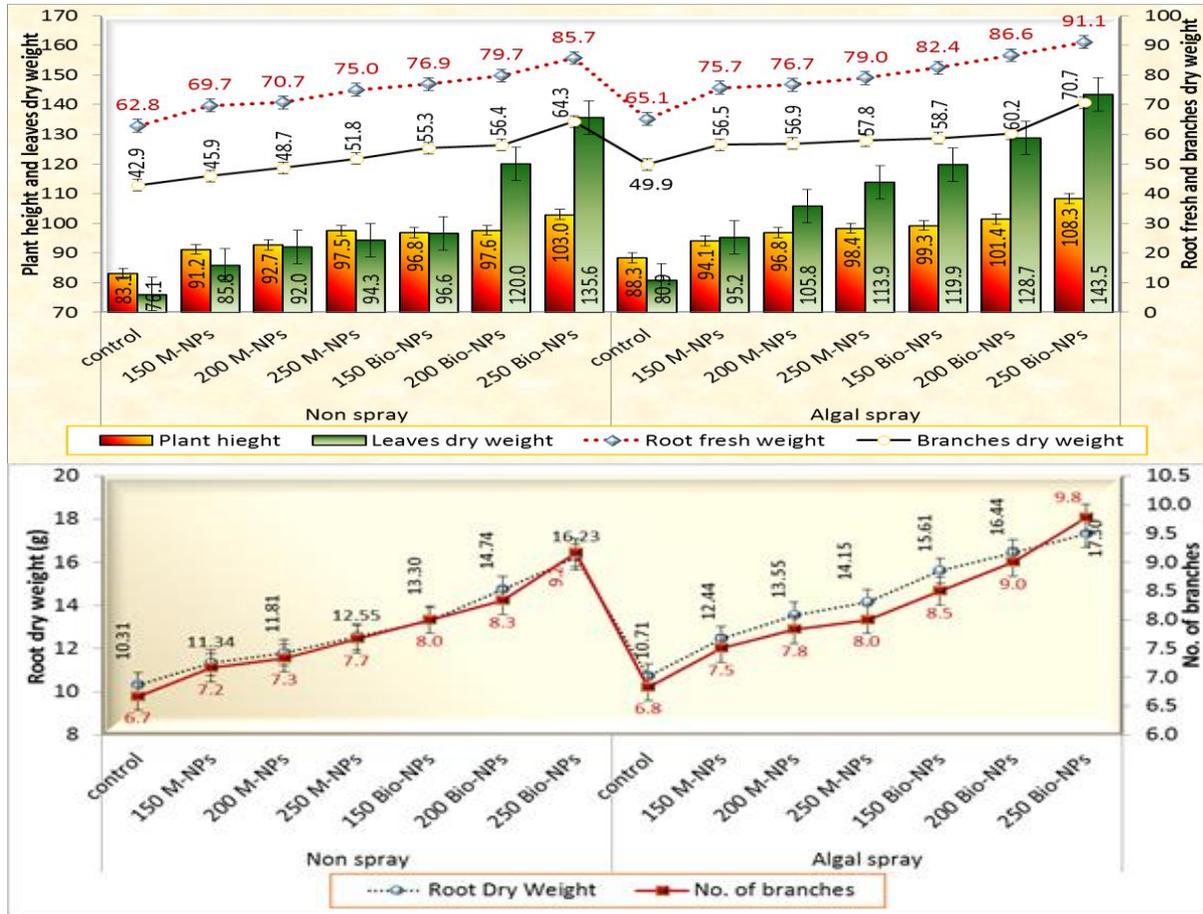


Figure 3: (a). Vegetative growth characteristics on average of both seasons of tomato plants as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray interacted with algal sprayed or non-sprayed treatments.

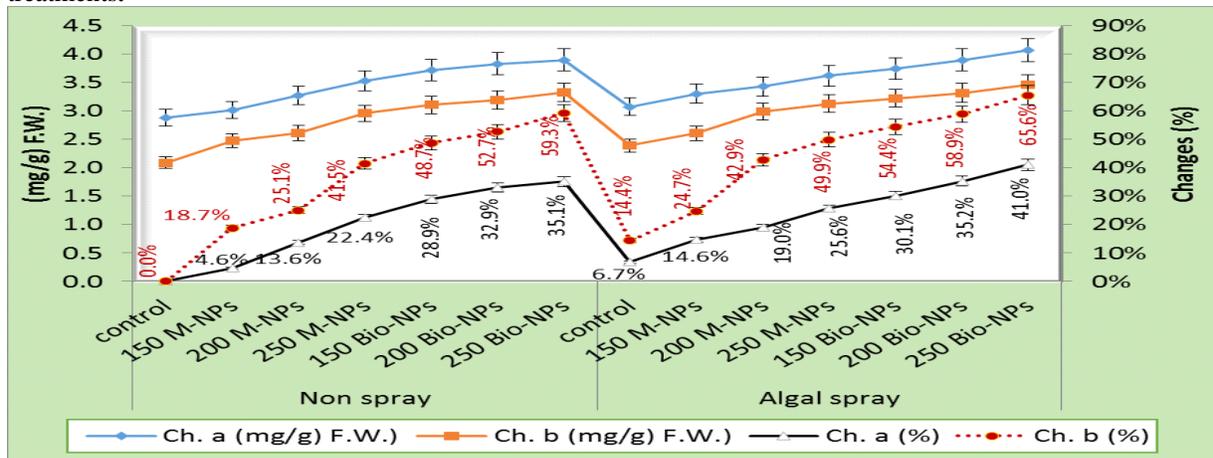


Figure 3: (b). Chlorophyll characteristics on average of both seasons of tomato plants as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray interacted with algal sprayed or non-sprayed treatments.

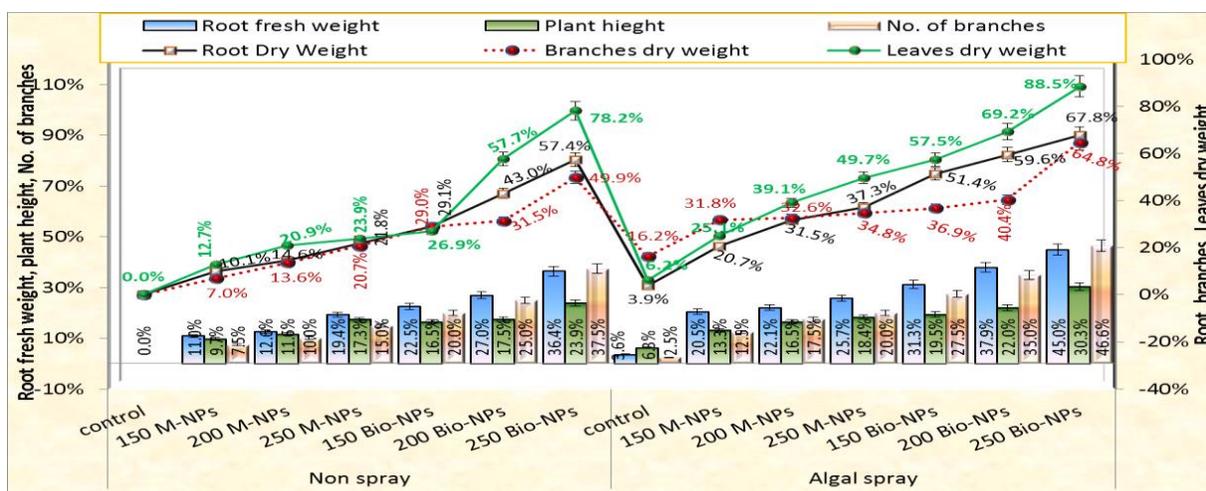


Figure 4. Change percentage of vegetative growth characteristics of tomato plants as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray interacted non-sprayed or with algal sprayed treatments.

3.3. Yield zinc content, total microbial and dehydrogenase's activities

3.3.1. Effect of algal spraying

The results presented in Table 3 indicated that algal spraying had a significant impact on yield traits, zinc content, and microbiological soil characteristics compared to the control (non-spray treatment) throughout the study seasons. The algal spraying treatment demonstrated a notable improvement over the control in all the mentioned traits, except for AFW-A, fruit zinc content, and dehydrogenase activity in the 2nd season.

3.3.2. Effect of Nanoparticles (NPs)

Data presented in Fig. (5) shows the effect of foliar applications, specifically two synthesis methods of nanoparticles in three different concentrations each, on fruit and yield parameters, Zn content in tomato organs, and the microbiological quality of soils. It is evident that all NPs (Met. or Bio.) foliar applications significantly increased AFW (A and B) (Fig. 5 a, and b), TY (Fig. 5 c), MY (Fig. 5 d), TMA (Fig. 5 e), and DHA (Fig. 5 f), as well as the contents of zinc in roots (Fig. 5 g), leaves (Fig. 5 h), and fruits (Fig. 5 i) compared with the control, except for the three metallic NPs concentrations for AFW-A and DHA; 150 ppm Met-NPs for total yield, TMA, root, and leaves Zn content in both

seasons; 200 and 150 ppm Met-NPs for total and marketable yields, respectively in the 1st season, as well as 150 ppm Met-NPs for fruit Zn in the 2nd season. However, it was observed that the high concentrations (250 ppm) of the two synthesis procedures of nanoparticles solutions provided a significantly higher enhancement than, or equal to, other concentrations (100 and 200 ppm) of the same ZnONPs procedure in all traits, except for AFW-B with no significant differences between the highest concentration of Met-NPs (250 ppm) and the lowest one of Bio-NPs (150 ppm) in all traits in both seasons, except for AFW-B and fruit zinc contents in both seasons and the 2nd season, respectively, in which 150 ppm bio-NPs exhibited the significantly highest increase.

Spraying metallic and bio-nanoparticles increased all traits, i.e., Leaves Zn, Fruit Zn, Root Zn (Fig 6 a.), MY, TY, AFW-B, AFW-A (Fig.6 b), , TMA, and DHA Fig.6 c) up to 20.83%, 69.1%, 57.01%, 38.68%, 32.24%, 26.08%, 21.11%, 1280.54%, and 252.51%, respectively in average of both seasons, where the concentration 250 ppm Bio-NPs exhibited the highest increment relative to their control treatment. However, no significant differences between the three concentrations within the Met-NPs in TY and DHA as show in fig 5b, and 5c respectively,

traits in both seasons as well as Root Zn (Fig.6 a) and MY, AFW-A (Fig 6b) in 1st, 1st and 2nd

season, respectively. The minimum the traits were observed in untreated plants.

Table 3. Yield, average fruit weight and zinc content of tomato plants as well as microbiological quality of soils affected by alga spraying at 2022 and 2023 seasons

Characters	Treatments	Non-spray		*Algal spray		LSD 0.05 %	
		2022	2023	2022	2023	2022	2023
AFW-A		129.31	132.79	133.21	134.63	3.43	ns
AFW-B		73.54	76.84	72.28	77.21	1.31	0.33
TY		19.68	20.80	20.80	22.05	0.52	0.83
MY		17.22	18.50	18.17	19.64	0.39	0.75
Zn content in tomato organs							
Root Zn (mg/kg DW)		150.90	147.62	173.23	170.58	8.78	10.79
Leaves Zn (mg/kg DW)		38.30	35.97	47.00	46.82	6.94	7.50
Fruits Zn (mg/kg DW)		33.59	35.48	43.81	40.02	6.61	ns
Microbiological quality of soils							
TMA (µg/g)		0.35	0.40	0.56	0.62	0.16	0.17
DHA (µmol/g)		0.08	0.11	0.16	0.20	0.07	ns

*Aqueous extract of *S. obliquus* microalga. AFW-A, B: average fruit weight grade A and B; TY and MY: total and marketable yield, DW: dry weight. TMA, DHA: Total microbial and dehydrogenases activity.

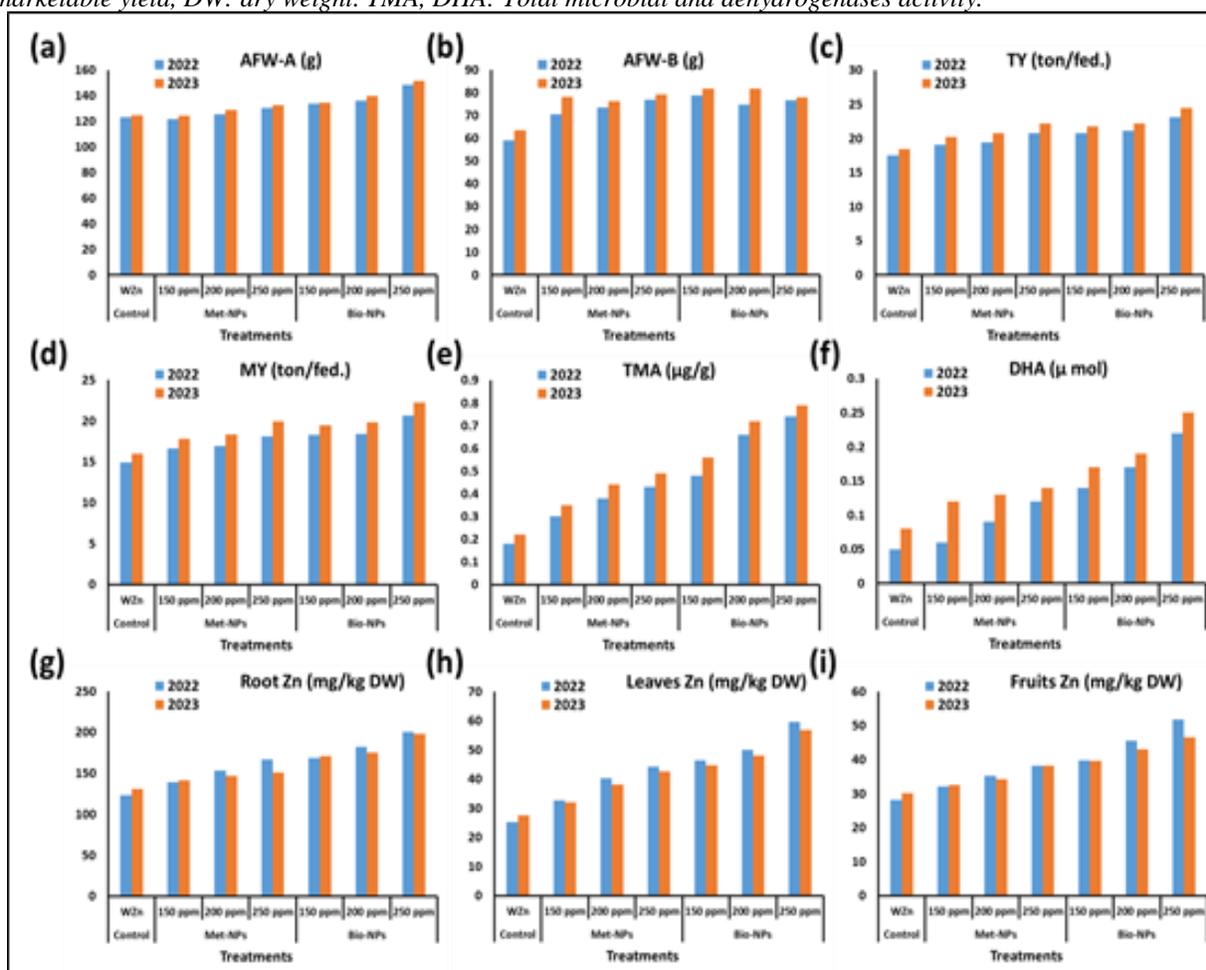


Figure 5. Effect of different metallic (Met-NPs) or biological (Bio-NPs) ZnO-nanoparticles foliar spray combined with algal sprayed as well as non-sprayed treatments (WZn: without zinc application) for tomato plants on average fruit weight grade A and B (AFW-A, B) (a,b), total yield (TY) (c), marketable yield (MY) (d), Total microbial activities (TMA) (e), total dehydrogenases activities (DHA) (f), average zinc content of roots (g), leaves (h), and fruits (i).

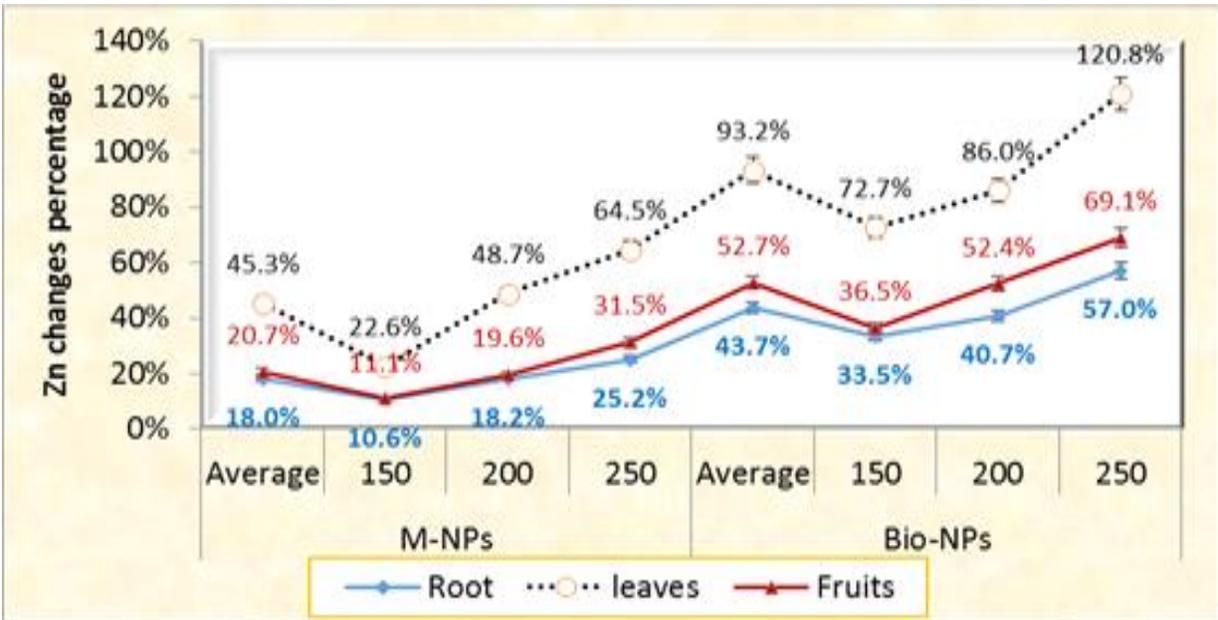


Figure 6: (a). Average, and percentage of root, leaves, and fruits zinc content of tomato plants as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spraying average two on seasons.

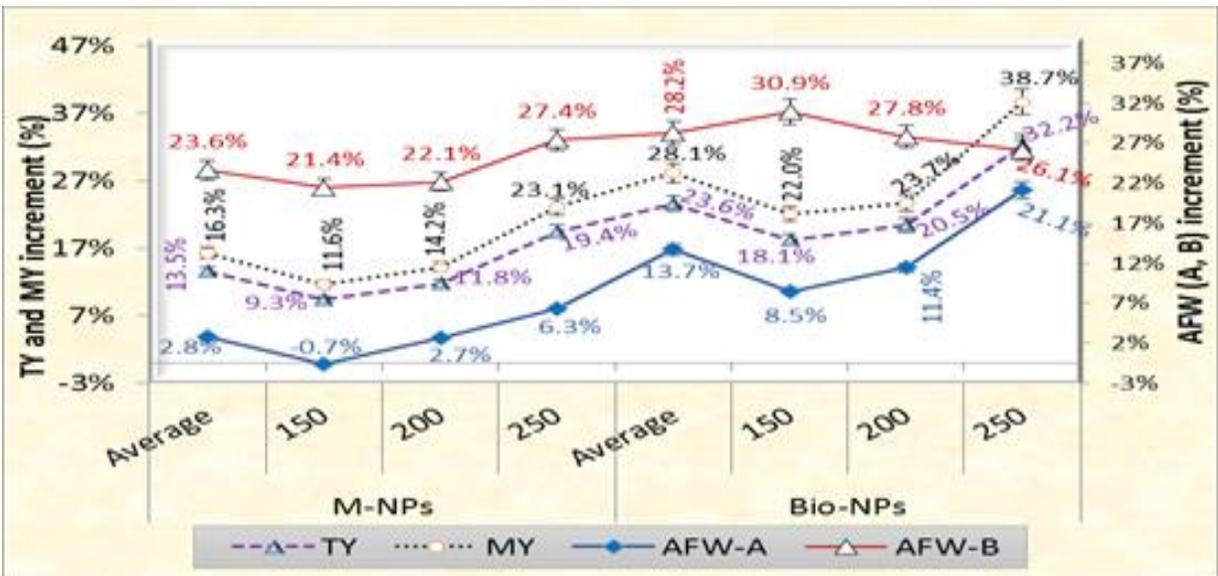


Figure 6: (b). Average, and percentage of total yield (TY), marketable yield (MY), and average tomato fruit weights (AFW-A & B) of tomato plants as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO nanoparticles foliar spraying average two on seasons.

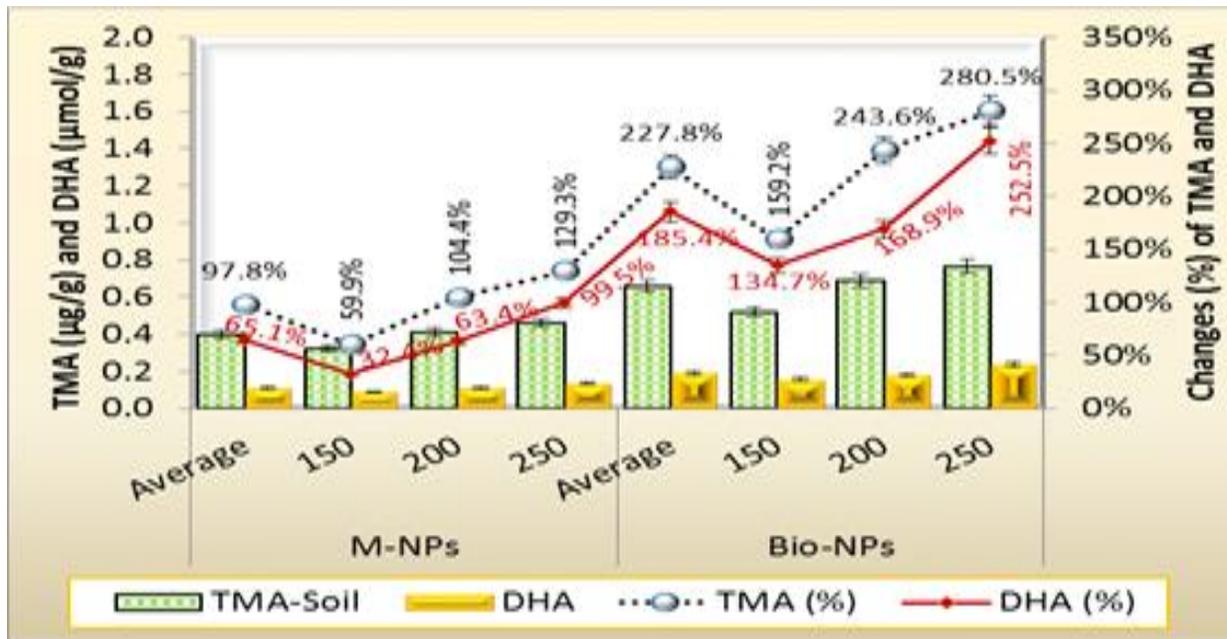


Figure 6: (c). Average, and percentage of total microbial activity (TMA) and dehydrogenase activity (DHA) in soils as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spraying average two on seasons

3.3.3. Interaction effect

Interaction effects between algal spraying and ZnO-NPs foliar spray are presented in Figures 7a, 7b, 7c on Zn content of tomato organs, fruit and yield parameters, as well as microbiological quality of soils.

A maximum value for root zinc, leaf zinc and fruit zinc (Fig. 7a), TY, MY (Fig 7b), TMA, DHA (Fig. 7c) traits was seen in plants treated with alga extract (AlgSp) plus 250 ppm Bio-NPs. This led to an increment percentage (as an average of both seasons) by 182.7%, 67.3.8%, 65.3% (Fig. 7a), 32.2, 20.9 (Fig 7b), 369.4%, and 298.8% (Fig 7c) respectively, over the control treatments (control without alga \times control without ZnONPs) (Fig. 2 and 3), which was followed by 250 BioZnONPs \times non-spray with no significant differences between them for TY and MY as well as 200 ppm BioZnONPs \times AlgSp for TMA, DHA and zinc contents of plant organs. On the other hand, the heaviest fruit was seen in AFW-A and AFW-B at 250 and 150 ppm Bio-ZnONPs without algal

spraying, respectively, followed by 250 ppm BioZnONPs \times AlgSp.

Figure 8 presents the UPGMA tree diagram generated by cluster analysis. In general, it shows two large classes (low and high LDW) included four groups. 1st group included both AlgSp-C and NonSp-C treatments which exhibited low values in all studied traits. Moreover, each of T1, T2, T3 and T4 in non-alga spray (NonSp) treatments as well as T1 in AlgSp treatment, also formed the 2nd group of the 1st class which has Low TMA and DHA. The third group (in 2nd class of high LDW which has medium to high values in all traits) included NonSpT5 (Highest AFW-B) and T6 (highest AFW-A and total yield) as well as AlgSp T2 (high AFW-B) in addition to both AlgSpT3 and T4 (exhibited high TMA, DHA and zinc contents in the three plant organs). Forth group which showed the highest values of all traits included both AlgSp T5 (200 ppm ZnO-NPs) and T6 (250 ppm ZnO-NPs).

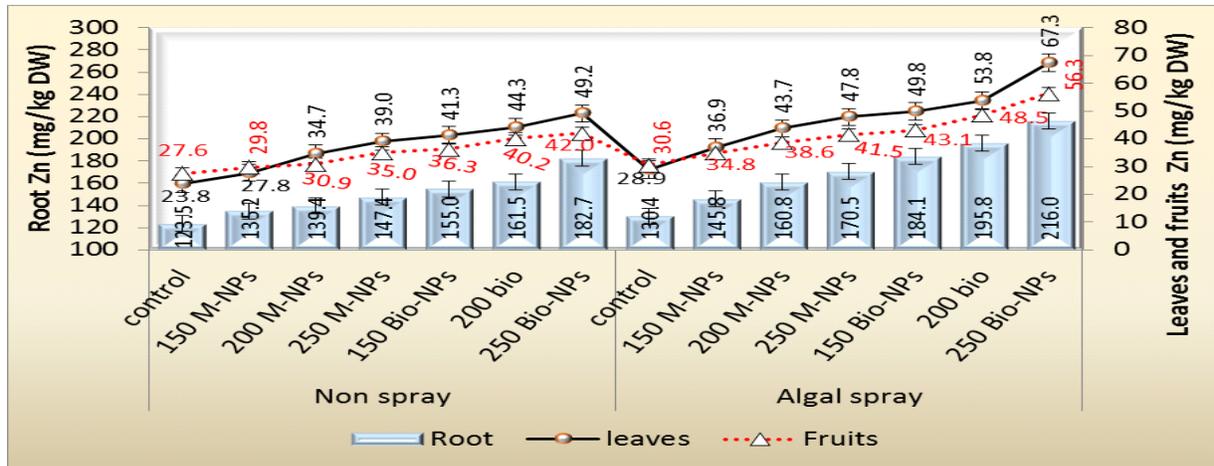


Figure 7: (a). Roots, leaves, and fruits zinc content of tomato plants on average of both seasons as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray interacted with algal sprayed or non-sprayed treatments.

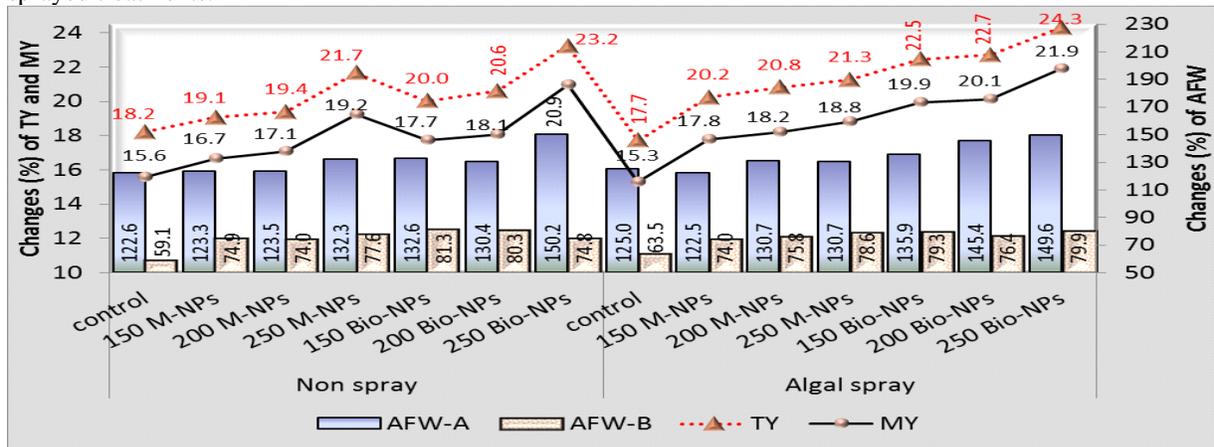


Figure 7: (b). Average fruit weight (A, and B) total yield, and marketable yield of tomato plants in average of both seasons as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray interacted with algal sprayed or non-sprayed treatments.

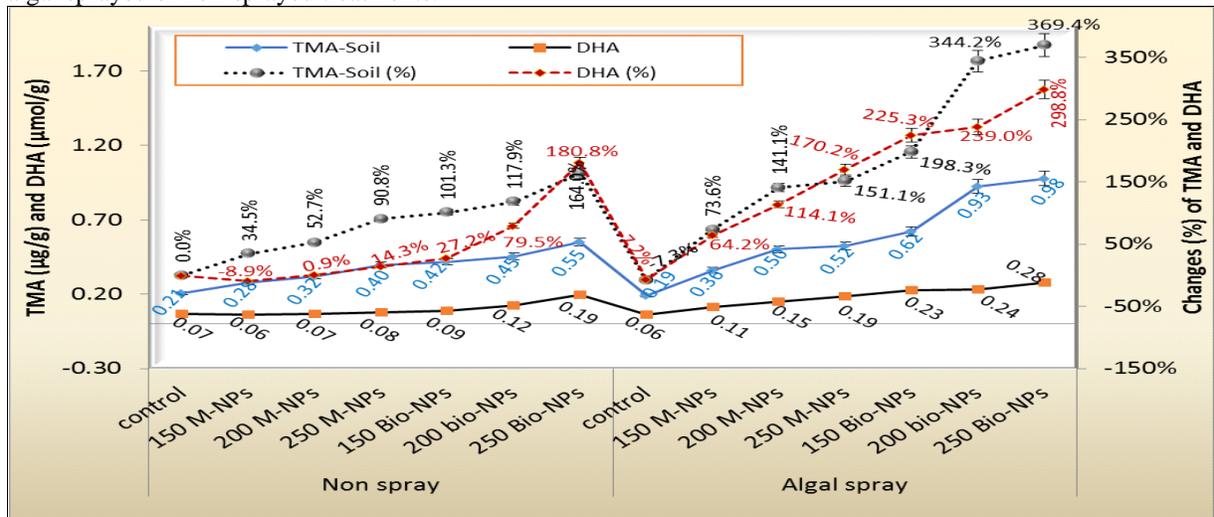


Figure 7: (c). Microbiological quality of soils (total microbial activity, and dehydrogenases) in average of both seasons as affected by Metallic (Met-NPs) or Biological (Bio-NPs) ZnO-nanoparticles foliar spray interacted with algal sprayed or non-sprayed treatments.

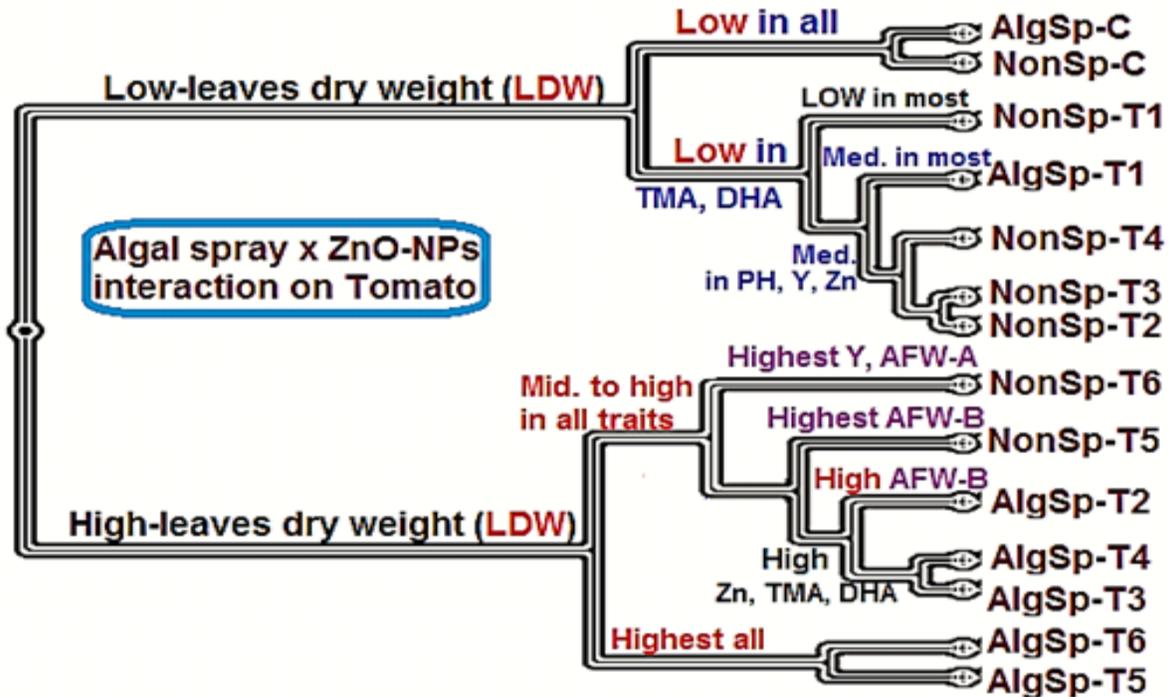


Figure 8. Dendrogram, using average linkage, for 14 treatments based on 13 vegetative and yield traits (where, AlgSp and Non-Sp: algal spray and non-spray, respectively; C: control; T1, T2 and T3: Metallic ZnO-NPs treatments; T4, T5 and T6: Bio ZnO-NPs treatments in 150, 200 and 250 ppm).

4. Discussion

These results according with those mentioned by Khan *et al.* (2009), Abou El-Yazied *et al.* (2012) and Gaafar (2014) where there was an increase in vegetative growth by the application of seaweed extract. On the other hand, Latique *et al.* (2013) found that seaweed application by foliar spray increases leaf pigment due to a decrease in chlorophyll degradation, which may be partly caused by betaines in algae extracts (Whapham *et al.*, 1993). Algal spraying treatment significantly surpassed the control in all fruit and yield abovementioned traits except AFW-A, fruit zinc content and dehydrogenase activity in the 2nd season. These results are in agreement with those mentioned by Amal *et al.* (2010), Gaafar (2014) Abo-sedera *et al.*, (2015), Uysal *et al.* (2015) and Vasileva *et al.* (2016) where there was an increase in the traits by the application of seaweed extract. The increase due to application of ZnO-NPs fertilizer may be due to the more availability of the element, which exerted beneficial effect on the plant (Farooq *et al.*, 2023). Zinc is a crucial

plant micronutrient that controls plant growth and development. Zinc-oxide nanoparticles, or ZnO-NPs, release Zn. (Sharma *et al.*, 2013). Auxin, also known as indole-3-acetic acid, is synthesized with zinc and is crucial for cell division and growth (Begum *et al.*, 2016). These outcomes are in line with those of Rizwan *et al.* (2019), who also observed that applying ZnO-NPs greatly enhanced the development of plants helping in the plant metabolic activity through the supply of such important micronutrient in the early vigorous growth that also enhancing physiological activities and thus improves photosynthesis in plants. Such growth augmentation may be attributed to a complex interplay of variables, including increased mineral absorption, decreased oxidative stress, and activation of metabolic pathways involved in biomass accumulation (Venkatachalam *et al.*, 2017). Such growth augmentation may be attributed to a complex interplay of variables, including increased mineral absorption, decreased oxidative stress, and activation of

metabolic pathways involved in biomass accumulation (Venkatachalam *et al.*, 2017).

It is evident that every interaction treatment produced values for every characteristic that were either statistically comparable or increased compared to the corresponding control treatments (control without alga \times control without NPs), indicating the efficient role of the algal extract in an increasing organic matter in soil and reduce the negative impact of soil stress which increased yield of plants (El-Hefny, 2010 and Gad El-Hak *et al.*, 2012).

The beneficial effects of the applied treatments (interaction of NPs and algal spray treatments) may be explained due to the nutritional status of plants greatly affects their ability to adapt to surrounding environmental conditions, and the increase in plant growth may be attributed to the valuable effects on stimulating the meristematic activity, for producing more tissues and organs, and cell enlargement, in addition to its vital contribution in several biochemical processes in the plant related to growth (Marschner, 1995) and may be, due to also, the functional role of zinc where, tightly related with membrane stability and integrity, signal transduction system (Lee, 2018). However, the indirect effects of ZnNPs on soil fertility include, Furthermore, directly; zinc may have various biochemical effects either at cell wall membrane level or in the cytoplasm (Daniel *et al.*, 2023; Denre *et al.*, 2014). The same trend was found by, El-Bassiony *et al.* (2010), El-Nemer *et al.* (2012), Ahmad *et al.* (2020) and Faizan *et al.* (2020).

Bio-NPs (nanoparticles green synthesis method) has been preferred over the years to the other method of synthesis due to various reasons such as cost-effectiveness, timesaving, utilization of less or non-toxic materials and waste and eco-friendliness nature of the method (Oluwaseun and Sarin, 2017).

5. Conclusion

Microalga extracts can be used to biologically convert mineral elements into their nanoforms, which have unique properties from elements in their ionic and chemically synthesized nanoforms. Application of biosynthesized Zn-NPs as well as the microalgal extracts as plant foliar fertilizer have stimulation effects on plant growth, and yield, as well as soil microbial activity. Applications of green biotechnology should be systematically expanded to produce environmentally friendly fertilizers that improve plant productivity, and save the environment and human health. Based on the findings of this study, we suggest the adoption of environmentally sustainable green nanotechnology approaches to enhance both tomato productivity and soil quality.

Authors' Contributions

All authors are contributed in this research

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest.

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