Alleviation of Salinity Stress by Ternary Se/CuO/ZnO Nanocomposite Synthesis by *Urtica urens* Biomass and their Antioxidant, Cytotoxic Properties

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Salinity stress is a major environmental constraint that adversely affects plant growth and their productivity. In this work, ternary selenium/copper oxide/zinc oxide nanocomposite (Se/CuO/ZnO NCs; TSCZ NCs) were synthesized for the first time using aqueous leaf extract of *Urtica urens* and evaluated for their ability to alleviate salinity stress in *Eruca sativa* plants. The synthesized nanoparticles were characterized using TEM, SAED, EDX, and XRD analyses, which confirmed the formation of predominantly spherical crystalline nanoparticles (average size of 58.71 ± 3.58 nm) and distinct elemental composition of Se, CuO, and ZnO. A pot experiment was conducted under normal and saline conditions (100 mM NaCl) using different concentrations of TSCZ NCs (up to 200 ppm). Salinity stress significantly reduced shoot and root growth, photosynthetic pigments, carbohydrate and protein contents, while increasing proline accumulation, antioxidant enzyme activities, and lipid peroxidation. Foliar application of TSCZ NCs partially mitigated these adverse effects, particularly at 100 ppm. The nanoparticles also modulated antioxidant defense enzymes in treated plants. In addition, TSCZ NCs exhibited strong antioxidant activity in the DPPH assay, reaching 89.4% scavenging at 1000 µg/mL, and demonstrated low cytotoxicity toward Vero normal cell lines. These findings suggest that Se/CuO/ZnO NCs may represent a promising nano-enabled strategy for improving salinity tolerance in plants.

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INTRODUCTION

The easiest way to appreciate the complexity of the abiotic global challenges in agricultural systems is to consider salinity (Devi *et al.* 2026). Water scarcity combined with the challenges of salinity in agricultural systems is a major constraint on productive capacity of agriculture (Petkova *et al.* 2019). The situation is critically important for crop productivity because non-salinized soil has been regarded as a prerequisite for irrigated land, and salinity is projected to affect more than 50% of irrigated land by 2050 (Khondoker *et al.* 2023). Salt can either directly or indirectly impact the growth and yield of crops in saline land. The first mechanism is that osmotic and water deficit salinity slows down the growth of crops as a result of slowing down the capacity of roots to take up water. The second mechanism is that the accumulation of salt in the leaf can negatively impact transpiration and increase the salinity level of the leaf, making leaf transpiration less active and thereby slowing down growth further (Hailu and Mehari 2021). Three major aspects of salt stress are: (a) reduction of water potential, (b) imbalance of ions resulting in perturbations of homeostasis, and (c) toxic ions (Arif *et al.* 2020). Salt stress impacts crops at different levels such as growth, biomass, and yield; it also impacts many physiological processes. Such damage leads to lower crop yields (Bhattacharya 2021; Balasubramaniam *et al.* 2023; Abid *et al.* 2026). Recent research has demonstrated that the effects of salt stress on different plant species can be mitigated by nanotechnology (Abid *et al.* 2026). Materials with dimensions between one nanometer and one hundred nanometers are referred to as nanoparticles. When compared to their bulk-sized counterparts, these materials are unique (Yadav *et al.* 2026). A nanocomposite is a multiphase solid in which nanoscale fillers (such as carbon nanotubes, clays, or metal oxides) are used to reinforce a bulk matrix (such as polymer, ceramic, or metal). These materials can significantly increase mechanical, thermal, electrical, and barrier properties at low filler loadings by utilizing fillers smaller than 100 nm (Amin *et al*. 2024).

The present study used nanocomposite of selenium/copper oxide/zinc oxide, which selenium is known to improve ROS scavenging and preserve membrane integrity (Liu *et al.* 2022), Plant-mediated reduction of selenium oxyanions (such as selenite or selenate) into elemental selenium (Se^0) is known to result in selenium nanoparticles (Se NPs). Naturally adsorbed biological macromolecules (such proteins and polysaccharides) that connect *via* covalent thiol bonds and non-covalent forces significantly stabilize them (Zambonino *et al.* 2023). Zinc and copper are necessary cofactors for numerous antioxidant and photosynthetic enzymes (Osman *et al.* 2026). Excess Na^+ causes oxidative burst and ionic imbalance in saline environments, which results in membrane lipid peroxidation, chlorophyll degradation, and metabolic inhibition. By boosting antioxidant defense systems (such as CAT, POX, and PPO), lowering ROS buildup and MDA formation, stabilizing photosynthetic pigments, and promoting osmolyte control such as proline metabolism (Chakraborty *et al.* 2018), foliar-applied ternary Se/CuO/ZnO nanocomposite may mitigate these impacts (Bhati *et al.* 2026). Furthermore, compared to traditional mineral salts or crude plant extracts, nanoparticles may show better leaf penetration and regulated micronutrient release, resulting in longer-lasting physiological advantages (Khan *et al.* 2026). The oxidation state of selenium is particularly important because, according to earlier research, partial surface oxidation to SeO_x may contribute to progressive selenium bioavailability, but soluble Se(IV) species are typically less phytotoxic and more stable than Se_3 nanoparticles (Burmistrov *et al.* 2025). The main component of selenium nanoparticles (Se NPs) is elemental selenium in the zero-oxidation state (Se^0). Their distinct biocompatibility and antioxidant action are based on this zero-valent state, which offers substantially less toxicity than harmful inorganic forms like selenite (Se^{+4}) or selenate (Sampath *et al*. (2024). A biogenic ZnO/CuO/Se nanocomposite with an average size of 40.9 to 50.2 nm demonstrated strong anti-H. pylori activity and strong antioxidant properties (Alharbi *et al.* 2025).

Among all leafy vegetables enjoyed by humans globally, rocket (*Eruca sativa*) has a prominent position (Tripodi *et al.* 2025). Salt is an important environmental constraint that limits both vegetative growth and productivity, and this species shows specific responses to its effects (Cavalcante *et al.* 2025). In the literature regarding the other crops, the responses to the application of Se, Cu, and Zn nanoparticles have drawn a lot of interest concerning their growth- stimulating and stress alleviating effects (Pérez-Labrada *et al.* 2019; Mogazy and Hanafy 2022; Mishra *et al.* 2025). In regard to salinity stress, little is known of the effects of the combination of Se/CuO/ZnO in the form of a combined nanoparticle preparation for use as a soil amendment for rocket plants. It has not been resolved sufficiently how such systems might regulate growth and photosynthetic capacity, osmotic regulation, and oxidative stress. By combining these actions, unrecognized routes of stress mitigation could be revealed, and the scope for using nanoscale solutions for the sustainable cultivation of vegetables could be expanded. There has been growing interest in the use of nanoscale solutions for the mitigation of abiotic stress; however, the application of selenium-based/copper/zinc oxide nanoparticles in saline environments remains largely unexplored (Al-Khayri *et al.* 2023; Ochoa-Chaparro *et al.* 2026). In particular, the responses of this system to growth, the control of photosynthesis, osmotic regulation, and oxidative stress in the species rocket (*Eruca sativa*) has not been studied in detail. Therefore, the current study focused on the use of *Urtica urens* leaf extract for the biosynthesis of ternary Se/CuO/ZnO nanocomposite (TSCZ NCs) and the exposure of these nanoparticles to salinity stress to study the effects of these nanoparticles on the morphology, physiology, biochemistry, and stress mitigation in terms of their antioxidant activity and cytotoxicity.

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EXPERIMENTAL

Materials and their Preparation

Urtica urens leaves were gathered, rinsed three times with tap water to get rid of loose or water-soluble contaminants, left to air dry at room temperature, and then ground into a fine powder using a lab grinder to get uniform sizes. To create the extract, 10 g of the resultant powder was mixed with 100 mL of distilled water and shaken at 150 rpm for 60 minutes at 50 °C. After that, the mixture was centrifuged to create a transparent supernatant that was used as a catalyst to create ternary Se/CuO/ZnO NCs.

Green Synthesized TSCZ NCs

In order to create TSZC NCs, 5 mM of solutions (Na_2SeO_3, Zn $(CH_3COO)_2\cdot 2H_2O$, and Cu $(CH_3COO)_2\cdot H_2O$) were mixed at 1:1:1 proportion with the gathered aqueous extract (3:1, v/v) at pH 8 while stirring at 70 °C for 60 min done. During this, the plant extract is used to reduce metal precursors and create NPs, a reddish-brown hue appears. The evaporation of the resulting solution followed by a wash with deionized water and alcohol allowed to get rid of any contaminants, the residue was recovered. The collected the reddish-brown powder residue was then annealed for two hours at 200 °C. This powder was then kept in a falcon tube for the next procedures.

Characterization of TSCZ NCs

Utilizing transmission electron microscopy (JEOL-1010-TEM, Tokyo, Japan), the morphological characteristics (sizes and forms) of the produced NPs were identified. To identify the crystalline or amorphous structure, the SAED (Selected area electron diffraction) pattern was also gathered. Additional analysis using JSM6360LA energy dispersive X-ray diffraction (SEM-EDX, JEOL, Japan) with an acceleration voltage of 5 kv, revealed the chemical makeup of NPs. Also, XRD analysis was also performed.

Growth Conditions

This study employed rocket (*Eruca sativa*) plant seeds that had been thoroughly washed with sterile distilled water under aseptic circumstances after being sterilized on the surface for 15 min using 0.1% sodium hypochlorite (NaOCl). Eight treatment combinations with two salinity levels (0 and 100 mM NaCl) and four concentrations of selenium/copper oxide/zinc oxide nanocomposite (TSCZ NCs) at 0, 50, 100, and 200 ppm were used in the experiment. Five replications of each treatment were conducted. Each plastic pot held 6.0 kg of sandy loam soil and was filled with sterilized seeds. Each pot was seeded with ten seeds. After the seedlings, each pot was thinned and five healthy plants were kept. Forty-five days after seeding, morphological and biochemical investigations were performed.

Plant Analysis Techniques

Photosynthetic pigment determination

Fresh leaves were treated with 80% acetone to extract photosynthetic pigments, which were then measured spectrophotometrically at 470, 649, and 665 nm (Vernon and Seely 2014). The Smith (2013) method was used to calculate carotenoids and chlorophylls.

Metabolic contents estimation

The anthrone-sulfuric acid method (Umbriet *et al.* 1959) was used to measure total soluble carbohydrates, and the Bio-Rad test (Lowery 1951) was used to measure soluble proteins. A UV spectrophotometer was used for the measurements.

Free proline determination

In accordance with Bates *et al.* (1973), free proline was measured using the acid ninhydrin reaction and sulfosalicylic acid extraction. Following toluene phase separation, absorbance was measured at 520 nm.

Lipid peroxidation evaluation

Malondialdehyde (MDA) was measured using the thiobarbituric acid technique in order to evaluate lipid peroxidation (Zhang *et al.* 2015). MDA was reported as $\mu mol\ g^{-1}$ FW, and absorbance was measured at 450, 532, and 600 nm.

Enzymatic antioxidant activity assays

According to Mukherjee and Choudhuri (1983), the antioxidant enzymes were extracted from young leaves and terminal buds using phosphate buffer and centrifugation at low temperature. The supernatant served as the crude enzyme extract for enzyme assays.

Catalase

By tracking the breakdown of H_2O_2 at 240 nm for 60 seconds, catalase activity was determined. Under controlled reaction circumstances, activity was measured in accordance with Aebi (1983).

Peroxidase

The increase in absorbance at 470 nm in the presence of H_2O_2 was used to measure peroxidase activity. The assay was carried out in accordance with Bergmeyer *et al.* (1974).

Polyphenol oxidase

Using catechol as the substrate, polyphenol oxidase activity was assessed by measuring absorbance at 395 nm following incubation (Kar and Mishra 1976). The rate of change in optical density per gram of fresh weight was used to express enzyme activity.

DPPH test and cytotoxicity for TSCZ NCs

0.96 mL of the DPPH solution was combined with varying amounts of TSCZ NCs (1.95–1000 µg/mL). A UV-Vis spectrophotometer (PerkinElmer, Germany) was used to quantify the decrease in absorbance at 520 nm. Using the MTT assay method, the cytotoxicity of TSCZ NCs was examined in relation to Vero cells, a mammalian cell line.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's test in SPSS software. Differences were considered significant at $p \leq 0.05$ (Nowwar *et al.* 2023).

RESULTS AND DISCUSSION

Ternary selenium/copper oxide/zinc oxide nanocomposite (TSCZ NCs) mediated by *Urtica urens* mostly showed a spherical and semi-spherical particle morphology when viewed under TEM. The average size of the nanoparticles was 58.71±3.58 nm, as illustrated in Fig. 1a and 1b.

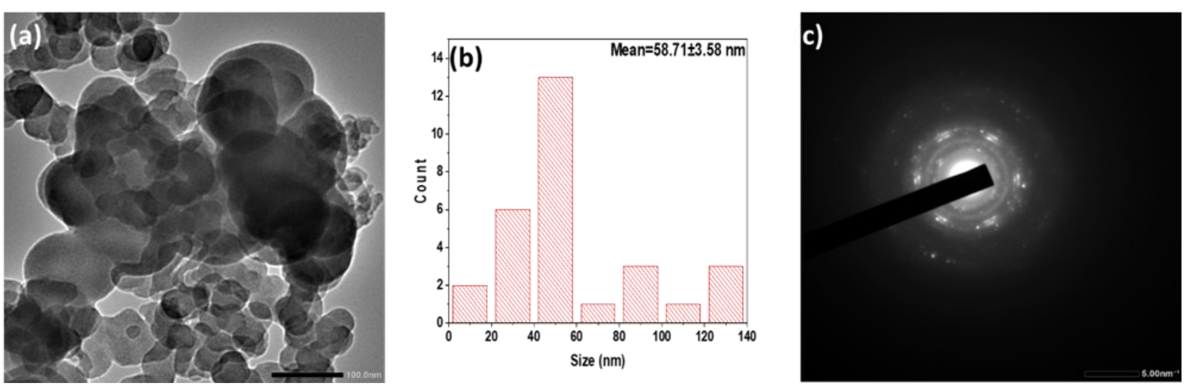


Fig. 1. Characterization of green-synthesized TSCZ NCs: The TEM and size distribution are displayed in A and B, and the SAED analysis for crystallinity detection is displayed in C.

The size of nanoparticles and morphology are critical parameters that define the physicochemical properties, biological activity, and scope of application. A comparable study used TEM to characterize spherical ternary Au/ZnO/CuO NPs (35 nm) prepared by aqueous extract of *Verbena officinalis* (Dobrucka *et al.* 2021).

The crystallinity of NPs can be assessed by the Selective Area Electron Diffraction (SAED) which is a technique used in electron microscopy. The concentric circular dark fringes observed in the SAED image of the TSCZ NCs, demonstrated the crystalline nature of the sample and the presence of the CuO, Se, and ZnO crystalline domains (Fig. 1c). Similar SAED results of the ternary ZnO/Se/CuO NPs synthesized by fungi revealed 8 concentric rings which were attributed to the crystalline structure of the tri-metallic elements (Hashem *et al.* 2023).

The elemental composition of the TSCZ NCs was confirmed by EDX spectroscopy, revealing the presence of selenium (Se) and the copper (Cu) and zinc (Zn) as well as oxygen (O). The main peaks for the TSCZ NCs, illustrated in Fig. 2, employees these elements. The Se, Cu, and Zn were found to be present in the decreasing order of Se> Cu>Zn. The atomic percent composition of TSCZ NCs showed Se, Cu, and Zn to be present at 14.2%, 10.3%, and 5.8 % respectively. The weight percent composition showed Se, Cu and Zn to be present at 34.7%, 20.2%, and 11.7%, respectively. A strong presence of oxygen was recorded at 58.9 % in atomic percent and at 29.2% in weight percent. This is tentatively attributed to the absorption of oxygen from the environment to the nanoparticles surface, along with the formation of cupric/copper and zinc oxides. The presence of carbon was attributed to the binding of proteins to the NCs surfaces, as these biomolecules are significant in stabilizing and capping the produced NCs (Kunwar *et al.* 2023).

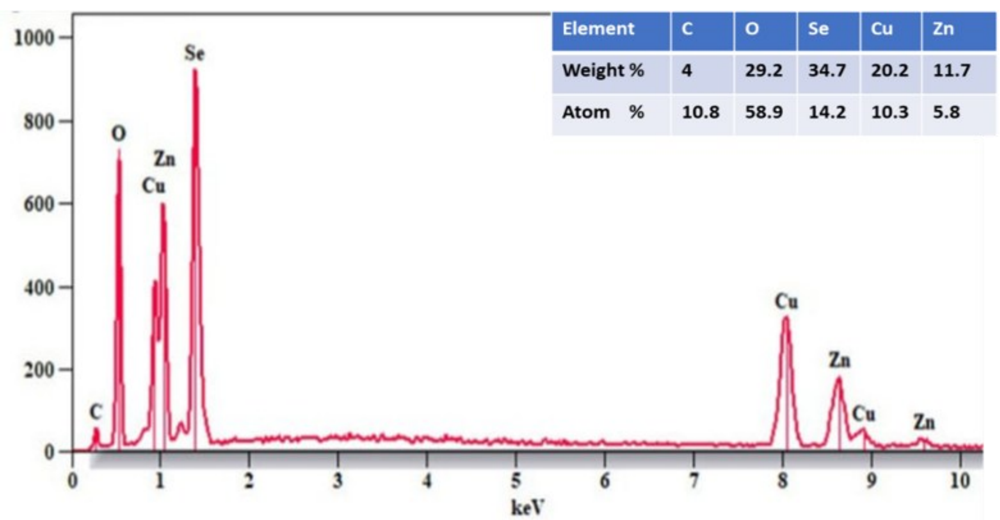


Fig. 2. EDX analysis of TSCZ NCs

The XRD pattern and peak assignments for the synthesized TSCZ NCs are shown in Fig. 3 and Table 1. XRD analysis revealed the variation in crystallite size for the different structures. For the ZnO phase, which is based on of the (100), (002), and (101) reflections, the average crystallite size was 20.1 ± 3.2 nm. For the copper oxide phase, which consists of the (111) and (200) reflections, the average crystallite size was 16.4 ± 2.8 nm. The Se-phase crystallite size was the smallest and was measured to be 13.7 ± 3.5 nm. The observed order of Se < CuO < ZnO may suggest that each of these phases nucleate and grow in a different way during the synthesis. However, without supporting studies on kinetics and surface chemistry, such a hypothesis is entirely speculative. Line broadening studies for the different phases showed small micro-strain in the lattice. The primary contributors to the line broadening for each of the phases were crystallite size and instrumental broadening

Table 1. Properties of XRD Analysis of TSCZ NCs

2θ (°)	Phase	Miller Indices	d-spacing (Å)	FWHM (°)	Intensity	Assignment
29.72	Se	(100)	3.004	0.62	Weak	Hexagonal selenium
31.77	ZnO	(100)	2.814	0.41	Very Strong	Wurtzite ZnO
34.42	ZnO	(002)	2.603	0.38	Very Strong	Wurtzite primary
36.25	ZnO	(101)	2.476	0.43	Strong	Wurtzite reflection
41.28	Se	(101)	2.185	0.68	Very Weak	Selenium minor peak
43.30	Cu	(111)	2.088	0.52	Medium-Weak	Metallic copper FCC
47.54	ZnO	(102)	1.911	0.46	Medium	Wurtzite reflection
50.43	Cu	(200)	1.808	0.58	Weak	Copper secondary peak
56.60	ZnO	(110)	1.625	0.44	Medium-Strong	Wurtzite reflection
62.86	ZnO	(103)	1.477	0.47	Medium	Wurtzite high-index

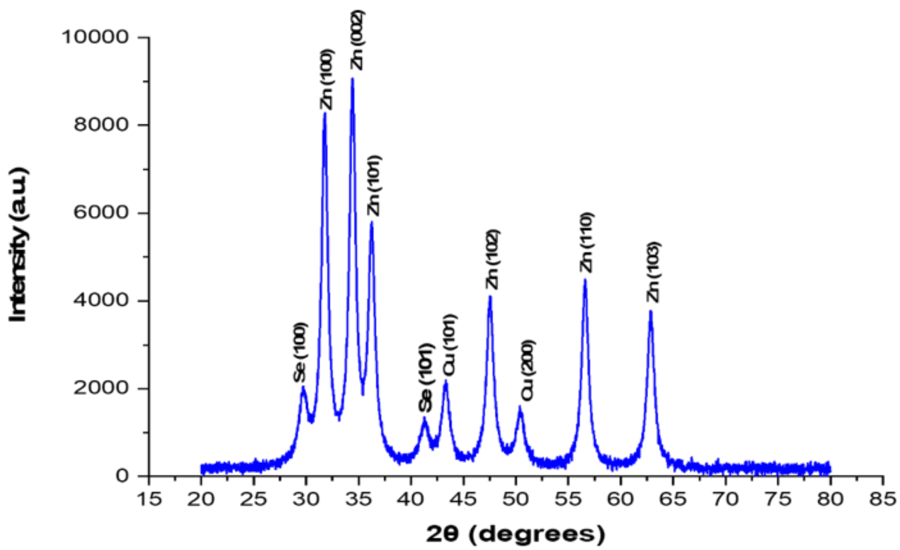


Fig. 3. XRD analysis of TSCZ NCs

Growth Parameters of Rocket Plant Under Salinity Stress

Elements like Se, Cu, and Zn, which may be deemed beneficial or essential, can be embedded in nanoparticles. These particles can promote positive alterations to stress response mechanisms in plants (Al-Khayri *et al.* 2023). Under salinity, CuO and ZnO NPs improved the growth of soybean, rice, and lemon balm (Ghasemian *et al.* 2021; Mishra *et al.* 2025; Chen *et al.* 2026).

Table 2 illustrates how salinity stress significantly reduced shoot and root lengths, shoot weight (dry and fresh), and root lengths as compared to the non-saline control. In contrast, when stressed plants were treated with 50, 100, and 200 ppm ternary Se/CuO/ZnO NCs, all the growth parameters showed highly significant improvement. Similar results were reported by Alamri *et al.* (2026) for oats, Riaz *et al.* (2026) for wheat, and Taj *et al.* (2024) for spinach. Selim *et al.* (2025) also investigated the biostimulant effect of bimetallic Se/ZnO NPs at 0, 50, 100, and 200 ppm on faba bean plants, and they reported that the treatment of bimetallic Se/ZnO NPs at 100 ppm resulted in the best values for root and shoot lengths, as well as shoot fresh and dry weights compared to the control.

Table 2. Effects of Ternary Se/CuO/ZnO NCs on Morphological Parameters of Rocket Plant Under Salinity Stress

Treatments		Shoot Length (cm)	Root Length (cm)	Fresh Weight of Shoot (g)	Dry Weight of Shoot (g)
Control	0 ppm	7.84±1.06cd	3.27±0.57a	0.36±0.07c	0.12±0.02ab
Salinity	100 ppm	5.34±0.95d	2.12±0.25a	0.24±0.05c	0.07±0.04b
Se/CuO/ZnO NCs	50 ppm	10.71±0.92a	3.79±0.15a	0.41±0.08b	0.13±0.01ab
	100 ppm	9.91±0.87ab	4.05±0.19a	0.62±0.13ab	0.16±0.01a
	200 ppm	8.99±2.40bc	4.13±1.23a	0.66±0.10a	0.14±0.05a
S+Se/CuO/ZnO NCs	50 ppm	5.75±0.57d	3.51±1.07a	0.31±0.08c	0.10±0.02ab
	100 ppm	7.56±1.26cd	3.85±0.95a	0.39±0.06bc	0.12±0.01ab
	200 ppm	6.61±0.99cd	3.83±0.91a	0.34±0.05c	0.10±0.02ab
HSD		1.52	0.95	0.10	0.03

Data represents means ± standard error (n=10). Different lowercase letters in the same species within columns indicate significant differences (P ≤ 0.05). HSD is honestly significant difference by post hoc-Tukey's test. S is salinity.

Photosynthetic Pigments

Rocket plants exposed to salt stress, unlike the control plants, showed significant depletion in total chlorophyll (a and b), total carotenoids. and total chlorophyll (b), as indicated in Fig. 4. From a mechanistic point of view, these losses are understood as at saline conditions, there is an increase in activity of chlorophyllase and destruction of chloroplasts (Fatahiyan *et al.* 2025). Similar results were reported in maize (Saravanan *et al.* 2026) and in lettuce (Kiremit *et al.* 2026).

In contrast to the stressed plants that were not treated, the application of TSCZ NCs increased total carotenoids, total pigments, and chlorophyll (a and b). When compared to the untreated stressed plants, the stressed rocket plants at 100 ppm of TSCZ NCs showed the greatest increase in total chlorophyll (a and b) and total carotenoids under salt. This increase in total chlorophylls and carotenoid may have resulted from the stability of the photosynthetic machinery by selenium and the activation of chlorophyll biosynthesis enzymes encouraged by copper and zinc (Rodríguez *et al.* 2018; Liu *et al.* 2022; Ochoa-Chaparro *et al.* 2026). Increased photosynthetic efficiency and biomass recovery are also made possible by pigment recovery. This was also noted in tomatoes (Hernández-Hernández *et al*. 2019) and rice (Mishra *et al*. 2025). Additionally, Alammari (2025) investigated the effects of exogenous Se NPs and Se/CuO NPs on salt-stressed maize and discovered that both enhanced the levels of carotenoids, chlorophyll (a), chlorophyll (b), and total chlorophyll (a + b) while reducing the detrimental effects of stress.

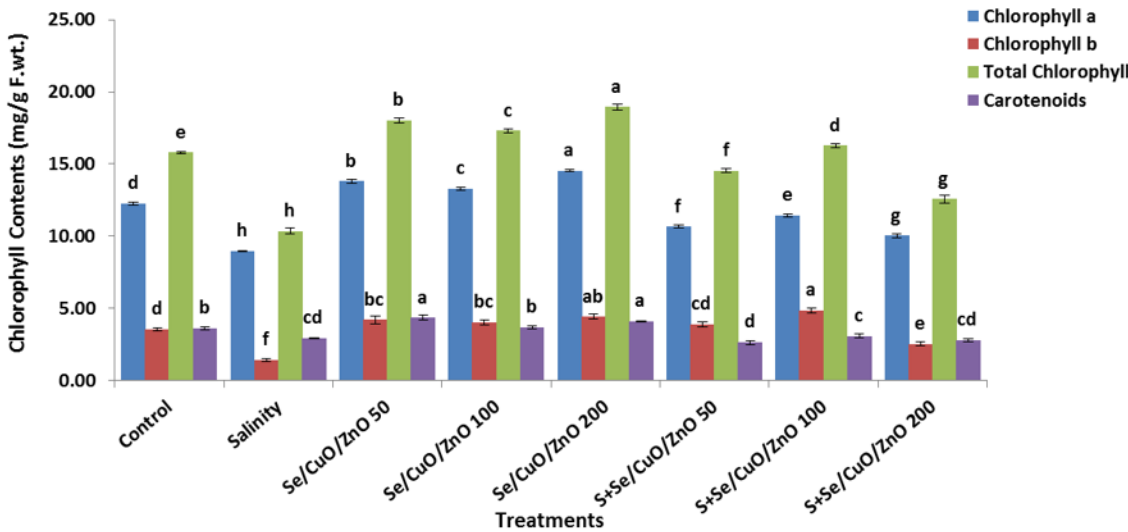


Fig. 4. The effect of Se/CuO/ZnO NCs on rocket plants under salt stress in terms of carotenoid levels (mg/g fresh weight) and chlorophylls (a, b, and a+b). The data shows the mean ± standard error (n=3). In the same bars, different lowercase letters denote significant differences ($P < 0.05$). According to the post hoc Tukey's test, HSD is an honestly significant difference. Salinity is represented by S.

Metabolic Contents

Under salinity stress, Rocket plants had significantly fewer primary metabolites (Fig. 5). A significant response to osmotic stress was shown by an 184% increase in free proline and 80% and 79% drop in protein and carbohydrate levels, respectively. According to Dichio *et al.* (2025), proline functions as a buffer to preserve cell turgor in this way, demonstrating adaptation to osmotic stress. These data are in agreement with Singh *et al.* (2025) and Rizzoli *et al.* (2025).

On the other hand, use of TSCZ NCs at different concentrations improved the biochemical makeup of plants. Under stressful conditions, plant treated with 100 ppm Se/CuO/ZnO NCs showed the biggest increase in carbohydrate contents by approximately 50%. TSCZ NCs at 200ppm showed the highest increase in protein contents by about 39% more than salt stress plants. On the other hand, by lowering proline buildup under stress by almost 91% compared to salt stress plant, 50 ppm Se/CuO/ZnO NCs showed osmotic stress mitigation. These outcomes show that these treatments were effective in reducing osmotic stress. These results are consistent with the findings that the relationship between Zn and Se enhances protein and sugar metabolism under stress (Gao *et al.* 2025). In another study, copper and zinc have been connected to the control of carbohydrate and protein synthesis (Sherefu and Zewide (2021). These data are also in agreement with Seleiman *et al.* (2023) in maize; El-Badri *et al.* (2021) in rapeseed and González-García *et al.* (2021) in bell pepper plants.

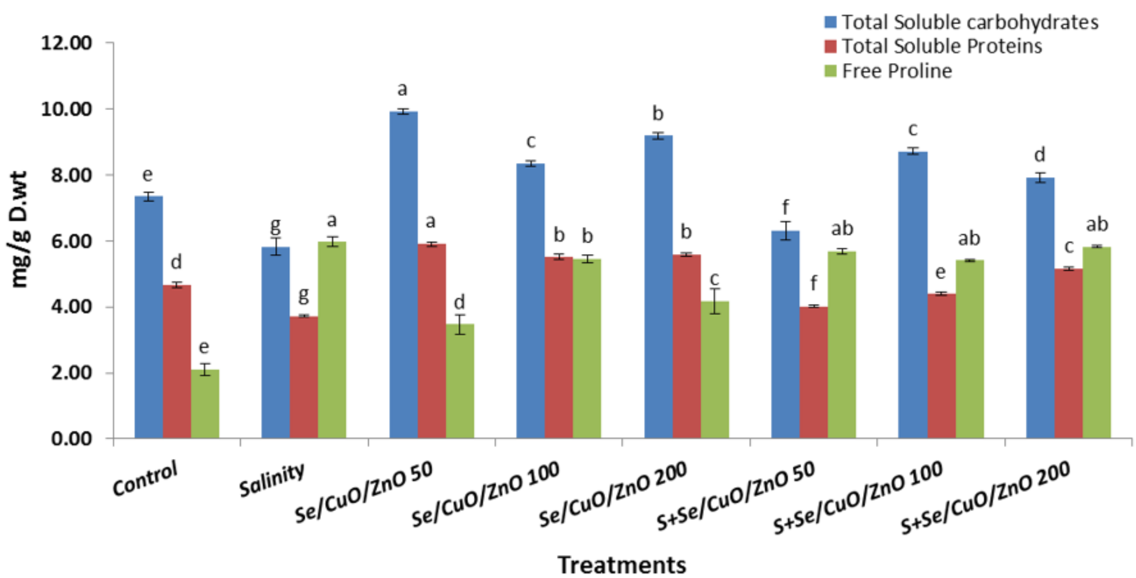
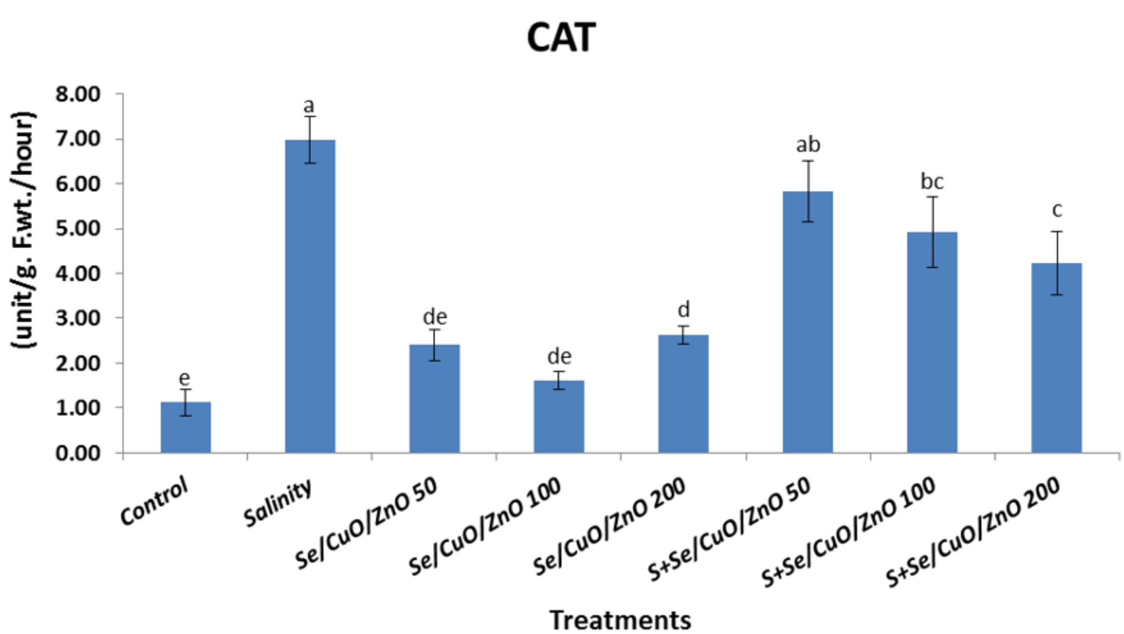
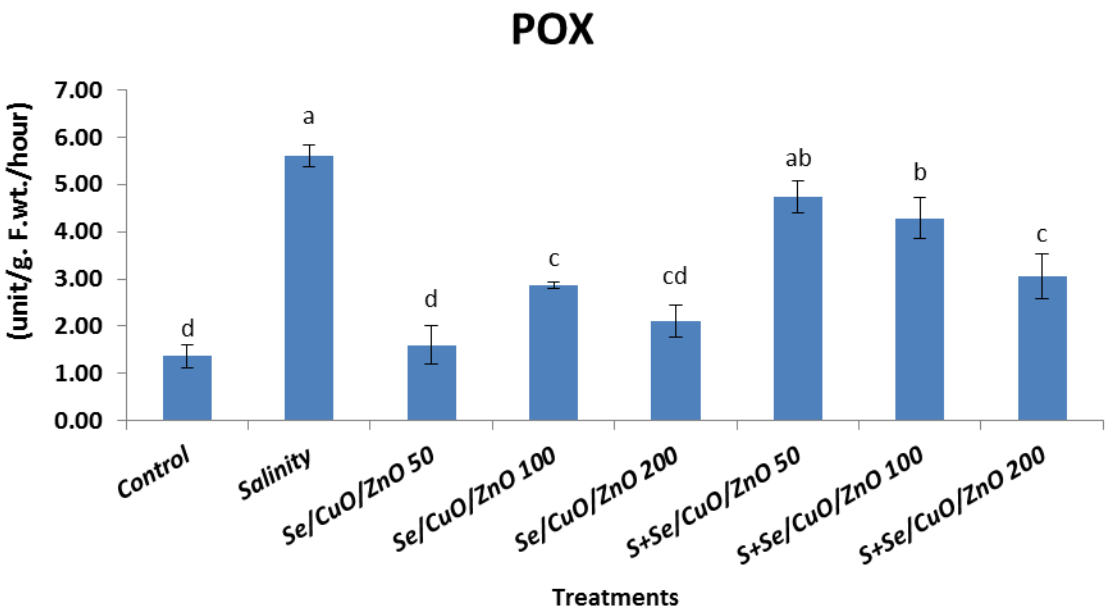


Fig. 5. Effects of Se/CuO/ZnO NCs on carbohydrate, protein, and proline contents (mg/g dry weight) of rocket plant under salinity stress

Enzymes Activities

This study shows a marked increase in the CAT, POX, and PPO enzymes in the rocket plant salt stress experiments (Fig. 6). The increased activity of antioxidant enzymes occurs as a defensive measure to increased levels of reactive oxygen species (ROS) in stressful conditions (Sachdev *et al.* 2021). These results are very similar to the most recent research by Osman *et al.* (2026) on faba beans and Shafiei *et al.* (2026) on cabbages.





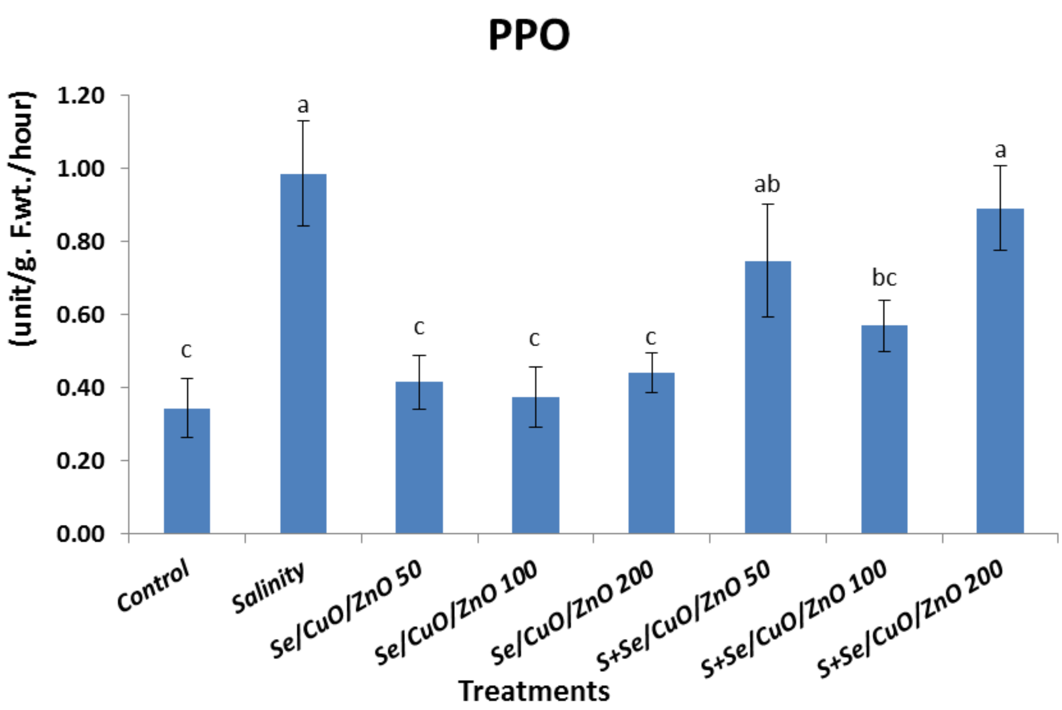


Fig. 6. Effects of Se/CuO/ZnO NCs on catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO) enzymes activities (unit/g. F.wt./hour) of rocket plant under salinity stress.

On the contrary, the Se/CuO/ZnO NCs, administered in the absence of salt stress, caused a small increase in antioxidant enzymes, which was not significantly different from the control. Within the different treatments, the Se/CuO/ZnO NCs caused the least amount of ROS of the salt-stressed plants, which suggests that the ROS levels in the plants should be very low. As evidenced in the works of Hasanuzzaman *et al.* (2024), Se, especially in nanoparticulate form, can enhance peroxidase enzyme activity as a control mechanism for ROS and assist in their detoxification. The results of the present experiments are similar to the previous experimental study which suggests that the Se, CuO, ZnO in nanoscale can control the enzyme activities and assist in the reduction of ROS in several varieties of crops exposed to salt stress as reported by Elsheery *et al.* (2025) on faba beans, Mishra *et al.* (2025) on rice, and Ghasemian *et al.* (2021) on *Melissa officinalis* plants.

Malondialdehyde Contents

Rocket leaves under salt stress collected a higher quantity of malondialdehyde (MDA) than the control (Fig. 7). Salt buildup in a cell's cytoplasm is a sign of ionic imbalance and hyperosmotic stress, both of which encourage the production of reactive oxygen species (ROS). As a result, membranes undergo oxidative peroxidation, which raises the MDA concentration (Ahmad *et al*. 2019). Other researchers have reported similar findings on other plant species: Tebini *et al*. (2025) reported comparable findings in Amaranthus caudatus treated to similar saline stress, while Khan *et al*. (2026) discovered higher levels of MDA in salt-stressed soybean.

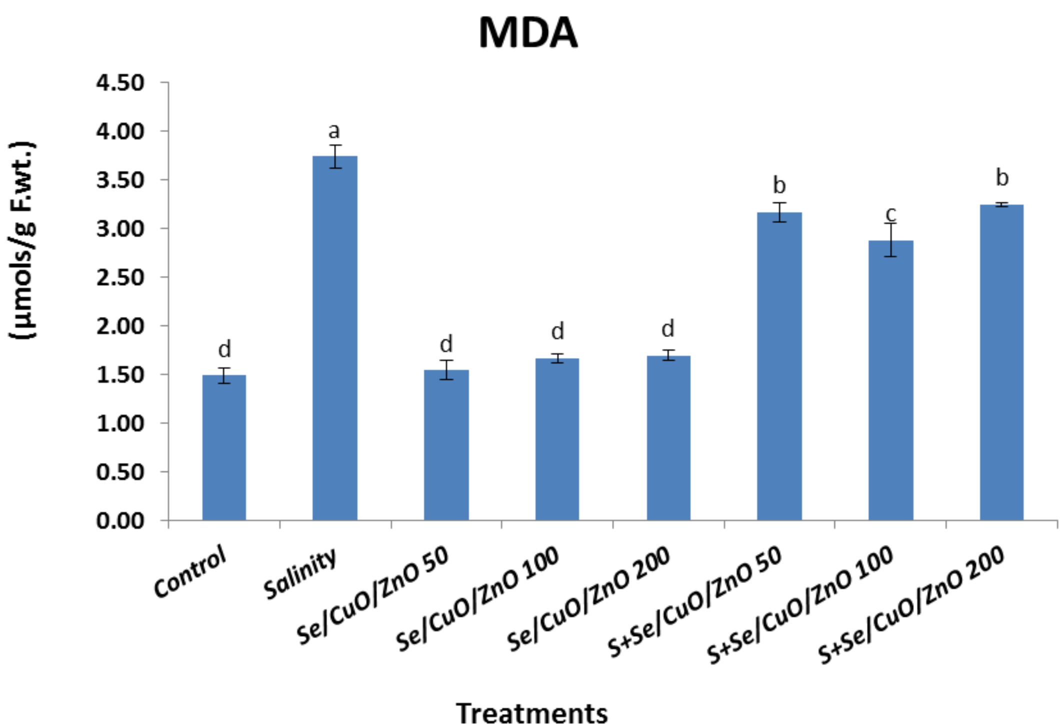


Fig. 7. Effects of TSCZ NCs on malondialdehyde (MDA) contents (µmols/g. F.wt.) of rocket plant under salinity stress. Data represents means ± standard error (n=3). Different lowercase letters in the same bars indicate significant differences ($P \leq 0.05$). HSD is honestly significant difference by post hoc-Tukey's test. S is salinity.

By lowering MDA and lipid peroxidation, TSCZ NCs helped salinized plants experience less oxidative stress. The rocket plants benefited from the application of TSCZ NCs because it also increased antioxidant enzyme activity, which quickly eliminated ROS and decreased oxidative and salinity stress on the plants, improving plant growth. These results were consistent with those of Mishra et al. (2025), who found that applying Se and ZnO NPs together greatly decreased salt stress in rice plants, enhancing their antioxidant defense and capacity to eliminate ROS. Lipid peroxidation also dramatically decreased during the trial. The use of Se, CuO, ZnO NPs in saline conditions also supported the findings of Talat *et al.* (2026) in mung bean, Alharbi *et al.* (2025) in cowpea, and Seleiman *et al.* (2023) in maize where the use of the nanoparticles reduced oxidative stress in the plants.

Antioxidant Properties of TSCZ NCs

Free radicals, often known as ROS, are unstable molecules that can function on their own. They are produced in the human body by oxidation processes or by exposing cells to harmful chemicals like radiation, toxins, and other compounds. The main constituents of cells, including DNA, amino acids, proteins, and lipids, are negatively impacted by this ROS. Finding new antioxidant molecules is therefore crucial to mitigating the negative effects of ROS. Here, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging, a common antioxidant assay technique, was used to assess the antioxidant capacity of biogenic TSCZ NCs. Instead of utilizing ascorbic acid, DPPH scavenging (%) was used to assess the antioxidant properties of TSCZ NCs (Table 3). With TSCZ NCs concentrations ranging from 1.95 to 1000 µg/mL, the DPPH radical efficiency rose dose-dependently from 17.7% to 89.4%. Another study on biogenic ternary Cu/Ag/Zn appeared that the scavenging rates were 68.3%, 37.3%, and 35.7% at concentrations of 1000, 500, and 50 µg mL^{-1} (Kunwar *et al.* 2023).

Table 3. DPPH Assessment of TZCS NCs

Concentration (µg/mL)	DPPH Scavenging (%)		HSD at 0.05
	Ternary Se/Cu/Zn NCs	Ascorbic acid	
1000	89.4 ± 0.15b	97.5 ± 0.14a	0.31
500	75.2 ± 0.25b	94.4 ± 0.18a	0.45
250	71.7 ± 0.15b	92.8 ± 0.15a	0.02
125	64.4 ± 0.18b	90.3 ± 0.08a	0.15
62.5	56.5 ± 0.01b	83.4 ± 0.07a	0.36
31.25	48.2 ± 0.05b	74.2 ± 0.02a	0.14
15.625	45.9 ± 0.01b	64.9 ± 0.01a	0.42
7.8125	32.7 ± 0.02b	56.4 ± 0.01a	0.65
3.9	26.5 ± 0.05b	49.8 ± 0.003a	0.38
1.95	17.7 ± 0.15b	44.2 ± 0.01a	0.45
IC_{50} (µg/mL)	54.21 ± 0.86a	3.72 ± 0.59b	0.19

Cytotoxicity of TSCZ NCs against Vero Cells

MTT assays were performed on Vero cells in order to determine whether TSCZ NCs exhibit very low cytotoxicity against normal cell lines. Results are shown in Table 4 and Fig. 8. The proliferation of the Vero cell line showed an inverse correlation with the nanoparticle concentration: at low concentrations the proliferation increased, whereas the proliferation decreased at high concentrations. Even at the highest concentration of 1000 µg mL^{-1}, the cell viability was 89.49%, and significantly increased with decreasing concentrations to 99.72% and 96.23% at 125 µg mL^{-1} and 250 µg mL^{-1}, respectively. These results, in the context of cytotoxicity, demonstrate that the synthesized TSCZ NCs did not affect normal cell line proliferation at the tested concentrations (250 and 500 µg mL^{-1}). In comparison, Amin *et al.* (2025) investigated the Se/ZnO/CuO NPs from *Nitraria retusa* and reported an IC50 on the WI38 normal cell line to be 294.9 ± 4.4 µg mL^{-1}.

Table 4. Cytotoxicity of TSCZ NCs against Vero Cells

Cells Type (Vero Cells)	ug/mL	Viability (%)	Toxicity (%)	IC_{50} (ug)
TSCZ NCs	0.00	100	0	0.00
	1000	89.49	10.50	
	500	91.63	8.36	
	250	96.23	3.76	
	125	99.72	0.27	
	62.5	100	0	
	31.25	100	0	

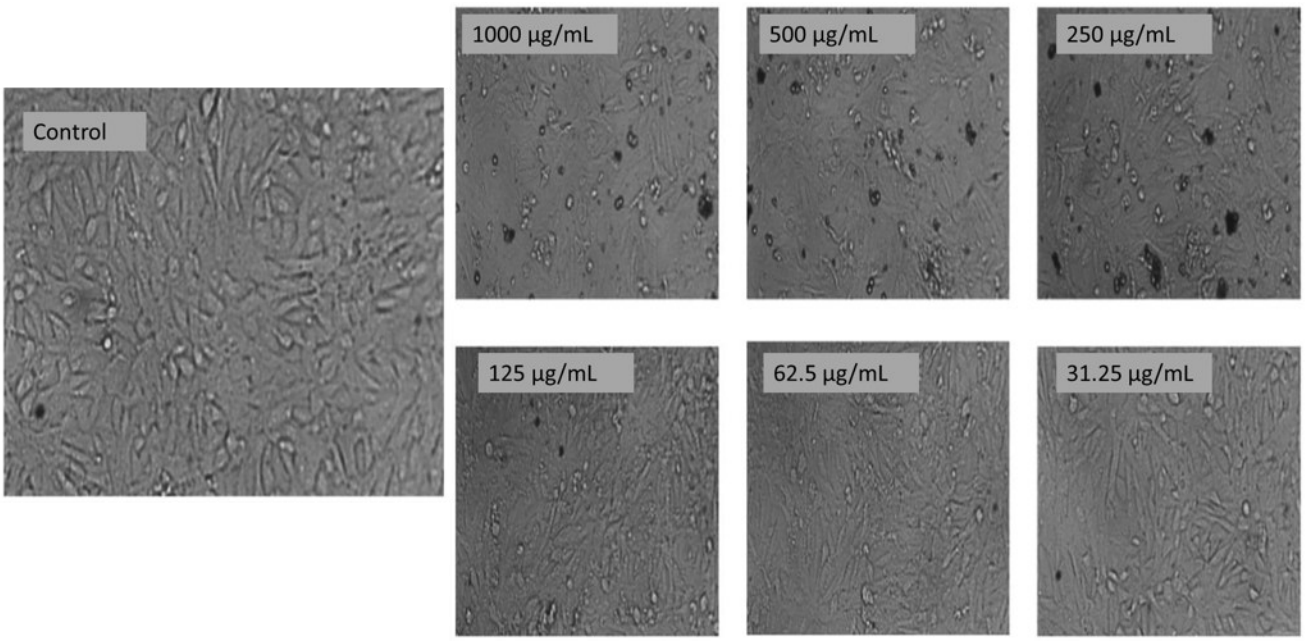


Fig. 8. Cytotoxicity of TSCZ NCs against Vero Cells

CONCLUSIONS

1. Successful green synthesis of biogenic ternary Se/CuO/ZnO nanocomposite by the leaf extract of *Urtica urens* was achieved. Structural characterization confirmed the formation of mainly spherical crystalline nanoparticles containing selenium, copper, zinc and oxygen associated phases.

2. Salinity stress had a significant inhibitory effect on the growth, photosynthetic pigments, metabolic constituents and oxidative balance of rocket (*E. sativa*) plants. However, foliar application of TSCZ NCs partially alleviated these negative effects through enhancing plant growth, restoring chlorophyll and carotenoid contents, improving carbohydrate and protein accumulation and reducing lipid peroxidation under saline conditions.

3. The observed modulation of antioxidant enzyme activities further suggests that TSCZ NCs contributed to improved oxidative-stress management in salt-stressed plants. The concentration-dependent response was observed as 100 ppm generally caused the most consistent physiological improvements among the tested concentrations under salinity stress.

4. Furthermore, the synthesized nanoparticles exhibited strong antioxidant activity and low cytotoxicity against Vero normal cell lines in the tested concentration range.

5. The results confirm the potential use of biogenic nanocomposite as nano-enabled plant bio stimulants for enhancing salinity tolerance. Nevertheless, additional studies are still needed to clarify the nanoparticle uptake, long-term phytotoxicity, environmental safety, and applicability at field scale before large-scale agricultural implementation.

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