Selenium/Copper Oxide Nanoparticles Prepared with *Urtica urens* Extract: Their Antimicrobial, Antioxidant, Antihemolytic, Anticoagulant, and Plant Growth Effects

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Urtica urens extract was applied for Se/CuO NPs synthesis with investigation of its biological activities and bio-stimulant qualities for *Zea mays* L. More antimicrobial activity was documented using Se/CuO NPs than *U. urens* extract against *C. albicans, E. coli, S. typhi, B. subtilis*, and *S. aureus*. Moreover less MIC and MBC values of Se/CuO NPs compared to *U. urens* extract against examined bacteria were recorded. Excellent antioxidant activity of Se/CuO NPs and *U. urens* extract was documented, with IC_{50} 4.2±0.41 and 5.12 ±0.19 µg/mL, respectively. Anti-hemolysis of *U. urens* extract was higher than Se/CuO NPs with IC_{50} 4.1±0.12 and 12.26±0.22 µg/mL, respectively. Anticoagulant potential of Se/CuO NPs was better than the *U. urens* extract *via* partial thromboplastin time (PPT) and prothrombin time (PT). Se/CuO NPs at 10 ppm caused the highest significant improvement in chlorophyll a and b of maize plants, but the highest value of carotenoids appeared at 20 ppm compared to the control and other treatments. The increase of shoot and root lengths was shown at 10 ppm of Se/CuO NPs by increasing 32.6 and 17.9%, respectively, compared to the control. Thus, novel bimetallic Se/CuO NPs synthesized using *U. urens* extract displayed antimicrobial, antioxidant, anticoagulant, anti-hemolysis, and plant biostimulant effects.

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INTRODUCTION

The advancement and utilization of nanotechnology have influenced various fields, including medicine and agriculture. It is critical to comprehend the disadvantages and advantages of using these products to ensure their sustainable and ideal use (El-Batal *et al*. 2023; Amin *et al*. 2024, 2025). Numerous investigations have focused on synthesizing nanoparticles (NPs) based on one metal, such as silver, copper, zinc, nickel, and selenium.

NPs of bimetals such as copper/zinc, zinc/selenium, zinc/gold, and iron/copper were studied by other investigations (Sharma and Dutta 2018; Al Abboud *et al*. 2024; Alghonaim *et al*. 2024a; Selim *et al*. 2025a,b), but there has been a lack of research concerning the multifunctional application of Se/Cu NPs. The synthesis of NPs was developed *via* incorporation of one or two bimetal NPs with organic polymers particularly in the medicinal and pharmacological applications (Abdelghany *et al*. 2023a; Al-Rajhi *et al*. 2024a,b). Numerous scientific papers have reported on the synthesis of NPs *via* physical, biological, and chemical approaches (Djamila *et al*. 2024). Although the main forms of harm brought on by NPs are cytotoxicity, genotoxicity, inflammation, and oxidative stress (Xuan *et al.* 2023), the biological method represents a safe alternative to the other applied methods for NPs synthesis. Synthesis *via* the extracts of microorganisms and plants has gained wide attention as a green, sustainable approach for creating a wide range of NPs (Abdelghany 2013; Qanash *et al*. 2023; Qanash *et al*. 2024). Moreover, the synthesis by plant extracts compared to microorganisms, are the greatest candidates for NPs synthesis because the extracts comprise several biomolecules such as polyphenols, alkaloids, and phenolic acids that serve as reducing agents to metal ions. Extracts can also be used as stabilizing the dispersible NPs subsequent to the process of synthesis (Abdelghany *et al*. 2018, 2023b). Many studies have demonstrated the safety and high efficiency of biogenic NPs as antioxidants and anticancer agents. For instance, Karimi and Mahdavi Shahri (2021) found that biogenic Ag NPs can act as an anticancer agent against MOLT-4 cell with IC50 at 0.011 compared to commercial anticancer Cisplatin with IC_{50} at 1.8 µm.

There are many ways to add micronutrients to plants, including by foliar application, seed priming, and soil drenching. However, priming the seeds has the benefit of facilitating easy access to nutrients during germination. It is also regarded as a cost-effective technique because so few nutrients are necessary for priming seeds (Farooq *et al*. 2021). Through improved photosynthetic pigments, antioxidant defense, osmotic changes, and membrane integrity, seed priming helps to improve drought tolerance (Saha *et al*. 2022).

Nanomaterials differs from bulk materials due to surface charge, chemical reactivity, and slow, controlled and adequate release (Otari *et al*. 2024). Bimetal NPs including selenium (Se) and copper (Cu) in doped form have been photosynthesized and applied in some biological activities. Se NPs are gaining main consideration due to their good bioavailability, low toxicity, and antioxidant, anticancer, and antimicrobial properties (Alghonaim *et al*. 2024b). Because Se NPs have a zero redox state (Se^0), they may have antioxidant properties and are lower in toxic effects (Hussein *et al*. 2019). The occurrence of SeNPs particularly with other metal NPs has become the highest target of many researchers who are focused on applications in agricultural, therapeutic, and nutrition fields. In the field of plant cultivation, plant growth promotion and enhancement of yields were recorded with using SeNPs (Safdar *et al*. 2023). Unique physicochemical properties were attributed to CuO NPs as documented in scientific studies and include catalytic, fungicidal, bactericidal, therapeutic, and optical features. Doping of different metals in nanocomposite form enhances the surface, biocompatibility, and physical, and chemical properties (Al-Rajhi *et al*. 2022a). Sharma and Dutta (2018) showed that the charge transfer properties were enhanced in CuO NPs when doped with selenium and at the same time improved the liberation of hydroxyl groups.

The maize plant is one of the most significant crops grown worldwide for the production of biofuel as well as food and feed (Piperno *et al*. 2009). Numerous studies have

shown that low-level selenium administration to plants enhances photosynthesis, promotes fruit growth, and quality (Feng *et al*. 2013). Copper is an essential element for plants, unlike selenium, and it plays a variety of roles in their metabolism, defense against oxidative stress, electron transport during photosynthesis and respiration, ethylene detection, and the biogenesis of the molybdenum cofactor (Yruela 2009). Application of Se and Cu NPs has been found to enhance the chlorophyll content, enzymes activity, vitamin C, flavonoids, and glutathione in tomato plants (Hernández-Hernández *et al*. 2019). The present paper aimed to synthesize Se/CuO NPs in doped form for the first time by using *Urtica urens* extract, and to evaluate its application in pharmacological and agricultural fields.

EXPERIMENTAL

Preparation of *Urtica urens* Plant Extract

The *Urtica urens* plant was gathered from the Department of Botany, College of Science at Al-Azhar University's botanical garden. The entire plant, or the gathered material, was left to dry in the shade for five days at room temperature after being cleaned with distilled deionized H_2O. Next, 100 mL of double-distilled deionized H_2O and 5 g of the whole plant powder were cooked for 20 min at 70 °C. This solution was further filtered through Whatman No. 1 filter paper and kept at 4 °C.

Synthesis of Bimetallic Se/CuO NPs

The precursors for the production of bimetallic Se/CuO NPs were copper sulphate (0.01 mM) and sodium selenite (1 mM). A stock solution of both precursor salts was combined in an equal volume of 60 mL, and the mixture was then heated for 10 min at 80 °C to complete the synthesis process. Thirty milliliters of plant extract were added with continuous mixing for one hour at 40 °C on a magnetic stirrer. The color of the bimetallic Se/CuO NP precipitates changed from green to black green. Centrifuging at 10,000 rpm for 20 minutes was used to gather these NPs, and the pellet was then completely cleaned in double-distilled deionized water three times to remove plant organic residues. It was then dried in a hot air oven at 80 °C and kept for later use at 4 °C.

Characterization the Biogenic Synthesis of Se/CuO NPs

Spectral techniques including energy-dispersive X-ray spectroscopy were employed to recognize the different chemical elements that comprised the nanoparticles. High resolution-transmission electron microscopy was used to observe the size and shape of nanoparticles. Fourier transform infrared spectroscopy (FTIR) was used to study the functional groups of the material after synthesis of Se/CuO NPS.

Antimicrobial Properties of Se/CuO NPs and *U. urens* Extract

By means of the well diffusion method, the activity of Se/CuO NPs and *U. urens* extract was determined against examined microorganisms (*Salmonella typhi* (ATCC 6539), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10221), and *Penicillium glabrum* (Op694171). Each tested microbe was cultured separately in a plate containing nutrient agar for bacteria, in addition to potato dextrose agar for fungi. With the use of a cork borer, 6-mm radius wells were prepared in the inoculated agar. Twenty-five mg/ mL of Se/CuO NPs and *U. urens* extract were poured into the wells. Ampicillin/Nystatin was applied to

inhibit bacteria/fungi as a standard control, while the negative control was DMSO. The appeared zone of inhibition around the well once the incubation time ended (24 h/37 °C for bacteria or 5 day/30 °C for fungi) was measured. The broth dilution technique was employed to detect the minimum inhibitory concentration (MIC) as described by Abdelghany *et al*. (2023b). Se/CuO NPs and *U. urens* extract (100 µL of 1 mg/mL) were injected into a 96-well microtiter plate and then incubated (24 h/37 °C). The dye of resazurin sodium was injected into the wells to observe the findings after the period incubation, and the shift of color to pink from purple was used to determine the MIC of Se/CuO NPs and *U. urens* extract.

Antioxidant Activity *via* 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Test

The DPPH test was carried out using various changes to the procedure of Al-Rajhi *et al*. (2022b). In 100 methanol, 20 mg of DPPH was dissolved to create the stock solution. Next, methanol was added to the DPPH solution to dilute it until the absorbance at 517 nm was between 0.68 and 0.76. For a whole day, the DPPH stock solution was covered with aluminum foil and left in the dark to inhibit the production of free radicals. Se/CuO NPs and *U. urens* extract ranging from 12.5 to 100 mg/mL was added to 3 mL, and the mixture was then incubated in the dark for 15 min. Ascorbic acid was utilized as the standard and the DPPH methanol reagent was used as the control solution in the absence of sample. After careful preparation, the mixture was kept at 30 °C for 30 min while it was dark.

On a spectrophotometer, the wavelength was recorded at 517 nm to detect the Se/CuO NPs and *U. urens* extract scavenging potential, which was calculated as follows,

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100 \quad (1)$$

where $A_{control}$ is the DPPH radical absorbance in methanol and A_{test} is the DPPH radical absorbance in tested samples.

The IC_{50} quantity, which represents the efficient dose of Se/CuO NPs and *U. urens* extract needed to scavenge DPPH radicals (at level of 50%), was recorded.

Assay of Hemolysis Inhibition by Se/CuO NPs and *U. urens* Extract

Three milliliters of recently drawn whole blood were taken in heparinized tubes from the corresponding author of the present study: Mohamed A. Amin who was in good health. The blood samples were then centrifuged for 10 min at 3000 rpm. After dissolving the collected pellets in the centrifuged tube in an amount of normal saline solution equal to the volume of supernatant, the pellets were suspended in an isotonic buffer solution made from Na_3PO_4 buffer (10 mM) at pH 7.4 to reconstitute the pellets at a volume of 40% v/v. Red blood cells that had been reconstituted (or re-suspended supernatant) were utilized in isotonic solution (IS). Separately, distilled water was utilized as a hypotonic solution (HS) to dissolve the *U. urens* extract and the used Se/CuO NPs. Se/CuO NPs and *U. urens* extract at varying concentrations (100 to 1000 µg/mL) were introduced into 5 mL centrifuge tubes containing HS (per dose). Erythrocyte suspension (0.1 mL) was then combined with the HS and the Se/CuO NPs and *U. urens* extract in separate tubes, gently mixed, and incubated at 37 C for 1 h. The mixture was centrifuged at 1300 g for 3 min. The spectrophotometer (Milton Roy) was used to measure the absorbance (OD) at 540 nm in order to determine the hemoglobin content in the supernatant. The percentage of hemolysis was calculated relative to 100% hemolysis in distilled water. The tube contained 5 mL of distilled water

was served as control, and another contained 5 mL of 200 g/mL of indomethacin served as a positive control (Al-Rajhi *et al*. 2022a). The provided equation was employed to assess the hemolysis inhibition % (HI) by the Se/CuO NPs and *U. urens* extract,

$$\mathrm{HI}(\%) = 1 - \frac{A_2 - A_1}{A_3 - A_1} \times 100 \quad (2)$$

where A_1 and A_2 are Se/CuO NPs or *U. urens* extract absorbance in IS and HS, correspondingly, while A_3 = Control absorbance in HS.

Anti-coagulation Properties of Se/CuO NPs and *U. urens* Extract

The anticoagulant activity of Se/CuO NPs and *U. urens* extract was assessed using *in vitro* classical coagulant assays, such as prothrombin time (PT) and activated partial thromboplastin time (APTT) tests (Fana *et al*. 2012). Nine parts of human blood were mixed with one part of sodium citrate (3.2%), centrifuged for 10 min at 5000 rpm, and the supernatant was collected. The PT and APTT assays were then performed on the blood. After mixing citrated normal human plasma with Se/CuO NPs and *U. urens* extract at varying dilutions, the mixture was incubated at 37 °C for 3 min. After 3 min of 37 °C incubation, 0.10 mL of the APTT reagent was added to the latex and plasma reaction mixture, and it was incubated for an additional 5 min at 37 °C. Following the incubation period, 0.10 mL of pre-incubated $CaCl_2$ (0.025 mol/L) was added and incubated for 3 min at 37 °C. The clotting time was then recorded. As stated in the APPT assay, PT was also measured; however, the reaction mixture was supplemented with a pre-incubated PT reagent (0.20 mL) at 37 °C for 3 min, and the clotting time was noted. Plasma only and Plasma with heparin were employed as negative and positive controls, respectively.

A Pot Experiment to Show the Effect of Different Concentration of Bimetallic Se/CuO NPS on Morphology and Pigments of Maize Plants

Zea mays L. var. Giza 368 (triple hybrid) seeds were provided by the Agriculture Ministry, Sakha Research Center, Kafr el-Sheikh, Egypt. Uniform seeds were planted in natural loamy soil conditions in a pot experiment containing 8 groups with 3 replicates representing the following treatments: control (soaking in tab water for 24 h), then 1, 5, 10, 20, 50, 100, 200 ppm of bimetallic Se/CuO NPS (soaking for 24 h). The plant samples were collected at 40 days old for morphological traits and assessment pigments of maize plants. Chlorophyll contents of were assessed using the method of Vernon and Seely (1996). Carotenoid contents of were estimated according to Lichtenthaler *et al*. (1981).

Statistics

With the Minitab 18 program, statistical assessment was computed. Post hoc analyses were conducted using Tukey's test (honest significant difference) with a significance level of $p < 0.05$. The standard deviation of the means plus the mean of three replicates make up each number. Values of the same letter in the same column do not differ significantly; nevertheless, post hoc Tukey's test results at $P < 0.05$ show significant differences between distinct lower-case letters in the same column

RESULTS AND DISCUSSION

Characterization of Biogenic Bimetallic Se/CuO NPS

Using EDX analysis, the basic elements of bimetallic Se/Cu NPs were identified both quantitatively and qualitatively. Figure 1A shows that the Se represented the main elemental content of the formed sample. The peaks of Cu ions appeared at bending energies of 0.8, 7.9 and 8.9 KeV but, the Se ion was detected in the sample at bending energy of 1.3, 11.5, and 12.6 KeV. The EDX analysis indicates the weight percentages of Se and Cu were 97.62, 1.1 respectively as well as with atomic percentages of 92.69 and 1.3 %. The presence of O ion with weight percentages of 1.28% and atomic percentages of 6.02%) could be related to the formation of CuO or the adsorption of O on the bimetallic surface from surrounding environments (Alshehri and Malik 2020).

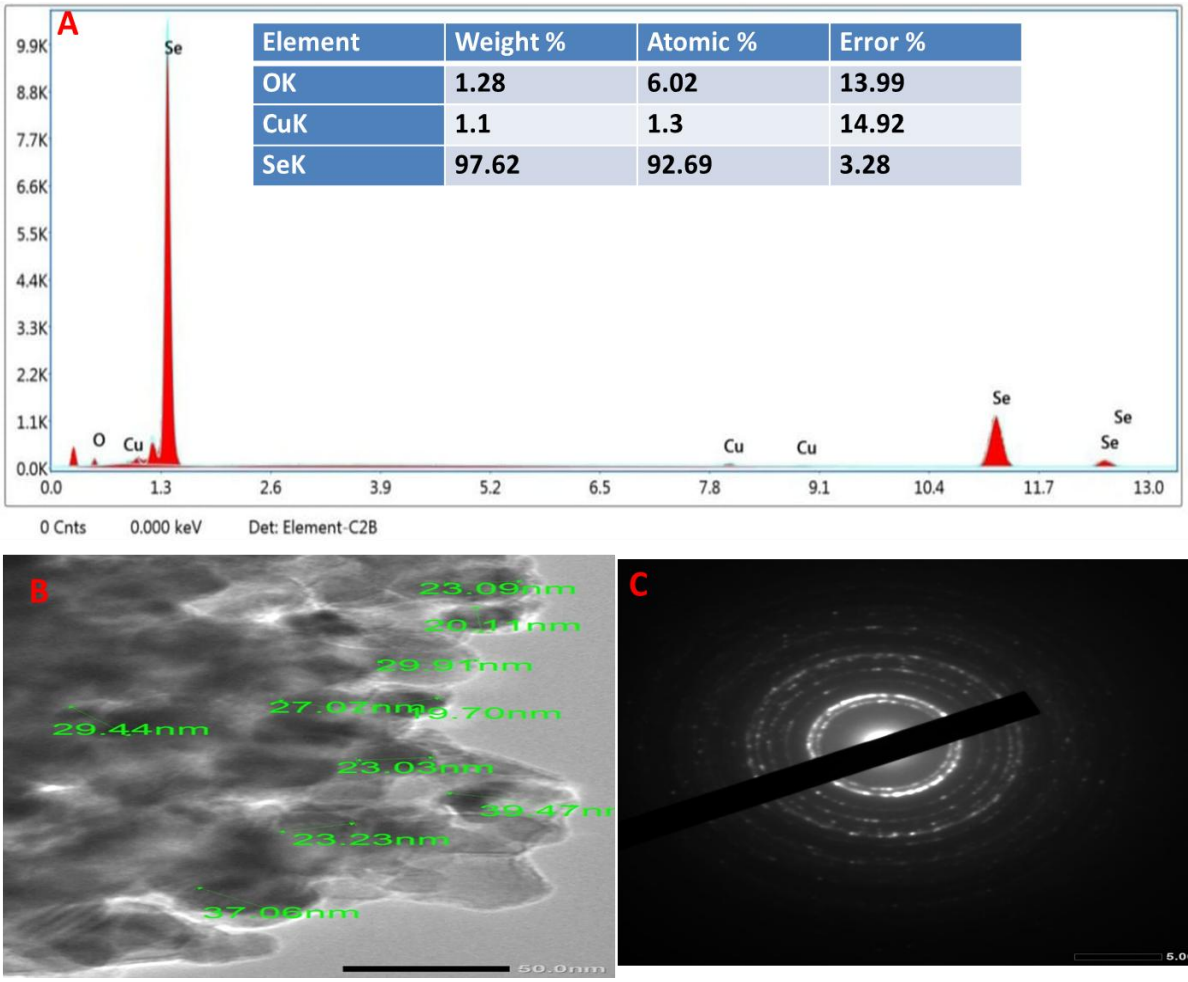

Fig. 1. Characterization of biogenic bimetalic Se/CuO NPS. A: EDX analysis, B showing the TEM and size distribution, C shown the SAED analysis for crystallinity detection

The TEM images show that the Se/CuO NPS were spherical or hemispherical with diameters about 20.4 to 55.9 nm (Fig. 2a). Similar to previously described elongated nanosnake-like particles, the nanoparticles tend to consolidate or come together (Huang *et al*. 2010). This suggests that a plant extract could be the agent of both forming the aggregates and stopping the hemispheric nanoparticles from completely changing morphologically. Recognition of the crystalline state of the produced nanoscale materials was aided by the technique known as selected area electron diffraction (SAED), which is associated with electron microscopy. Here, the bimetallic NP's SAED image revealed eight distinct and crisp rings that correspond to the component materials' crystallographic planes (Se and CuO) (Fig. 1C).

FT-IR was used to identify the functional groups found within the plant extract and their function in the reduction of metal precursor to generate Se/CuO -NPs, which causes new peaks to arise or the intensity of existing peaks to change (Fig. 2). A band at 3428 cm^{-1} was identified as the source of the O–H bending of phenolic substances; however, after the production of Se/CuO NPs with an equivalent intensity Hamza *et al*. (2020), the band moved to 3432. The carboxylic group (COO^{-}) vibration was assigned to the little band at 2356 cm^{-1}, which moved to 2361 cm^{-1} after synthesis of Se/CuO NPs with the similar intensity. The peak at 1606 cm^{-1} was assigned to the amide ($CONH^{-}$) group which shifted to 1652 cm^{-1} after synthesis of Se/CuO NPs. The peak at 1103 cm^{-1} revealed the presence of (O–H) stretching for alkyl group. The peaks positioned at around 613 and 492 cm^{-1} correspond to the characteristic stretching vibrations of Cu–O or SeO nanoparticles. Moreover, the band seen at 1050 cm^{-1} may be attributed to the broad range alkene group's C=C stretching, which aids in the formation and stability of Se-CuO NPs (Saeed *et al*. 2019). The presence of different functional groups within plant extract could be associated with amines, proteins, carbohydrates, amino acids, and other macromolecules that are in charge of Se/ZnO-/CuO-nanoalloy reduction, capping, and stabilization (Nguyen *et al*. 2023).

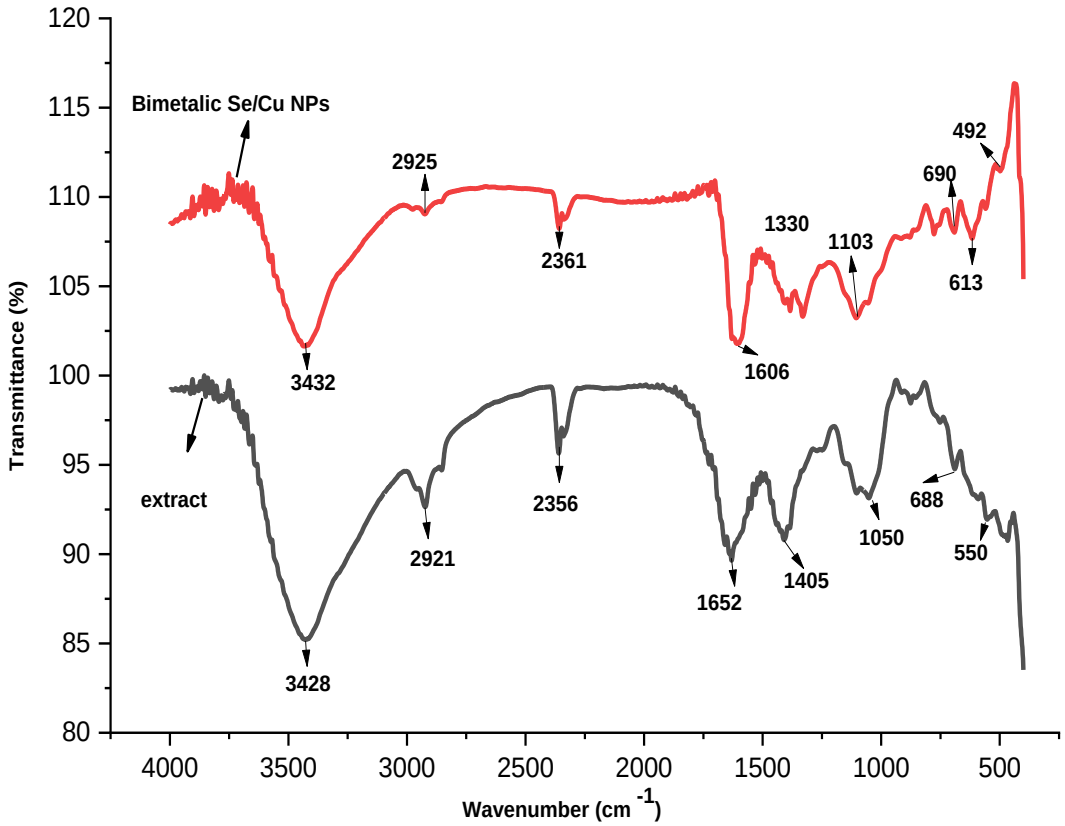

Fig. 2. FTIR of plant extract and bimetallic of Se/CuO NPS

Antimicrobial Activity of Se/CuO NPs and *U. urens* Extract

The apparent inhibition zones in Fig. 3 indicated that Se/CuO NPs and *U. urens* extract-mediated Se/CuO NPs possessed antimicrobial activities. Different levels of inhibition zones were recorded in Table 1 based on the tested microorganisms and tested sample, but generally Se/CuO NPs exhibited higher activity than extract. Growth of *C. albicans*, *E. coli*, *S. typhi*, *B. subtilis* and *S. aureus* were stopped with 31±0.48, 28±0.58, 26±0.69, 26±0.54, 27±0.74 mm of inhibition zones using Se/CuO NPs while its growth was stopped with 25±0.65, 26±1.25, 19±1.12, 24±0.98, and 25±0.28 mm of inhibition zones using the *U. urens* extract, respectively. The inhibitory action of Se/CuO NPs and *U. urens* extract was compared to standard antibiotic/antifungal which reflected significant differences in inhibition zone in case *C. albicans* with 2.65 HSD at 0.05. The Se/CuO NPs, *U. urens* extract showed antibacterial activity and inhibited C. albicans but didn't give antifungal activity against the filamentous fungus *P. glabrum*. This may be due to differences in wall structures. Significant differences appeared between Se/CuO NPs and standard antibiotic/antifungal at all tested bacteria and *C. albicans*. *P. glabrum* as filamentous fungus wasn't affected by *U. urens* extract or *U. urens* extract. Several bacterial species, namely *S. aureus*, *B. subtilis*, *S. typhi*, and *E. coli*, were inhibited by CuO NPs at 20 mg/ mL with maximum inhibition zone of 17.8 ± 0.4 mm (Dulta *et al*. 2022). In another study of Qanash *et al*. (2024), antimicrobial properties of ZnO NPs were enhanced when doped with SeONPs against different bacteria and fungi with inhibition zones of 28.33, 27.33, 27.67, 27.17, 27.5, 24.33, 23.33 and 18.17 mm against *S. aureus*, *B. subtilis*, *Bacillus cereus*, *P. aeruginosa*, *E. coli*, *S. typhi*, *C. albicans*, and *Aspergillus niger*. Se NPs

exhibited antimicrobial activity against *E. coli C. albicans*, *K. pneumoniae*, *S. aureus*, and *B. subtilis* (Alghonaim *et al*. 2024b). Regarding *U. urens* extract, their antimicrobial activity was reported in earlier investigation of (Mzid *et al*. 2017; Salem *et al.* 2021) against *C. albicans*, *S. aureus*, *B. subtilis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus*, and *Micrococcus luteus.* The size and structure of NPs have an impact on their activity in live cells. He and colleagues found that the size and form of the NPs affect the diffusion, dispersion, and response of the cells (He *et al*. 2010). For instance, the diameters of the produced CuO-NPs affected their antibacterial effectiveness against *B. subtilis* and *S. aureus* and *P. aeruginosa* and *E. coli* (Azam *et al.* 2012).

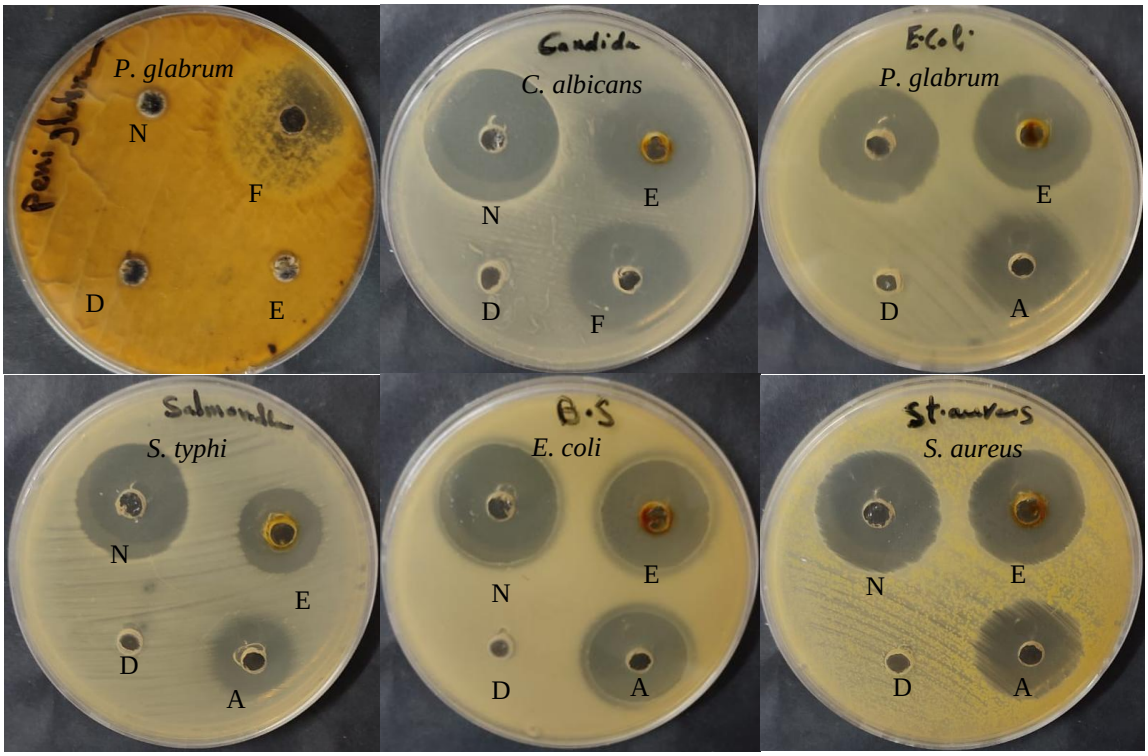

Fig. 3. Antimicrobial activity of Se/CuO NPs (N), *U. urens* extract (E), standard antibiotic (A) /antifungal (F), and negative control (D) against different microorganisms

Table 1. Antimicrobial Activity of Se/CuO NPs, *U. urens* extract *vs.* Control

Microorganism	Inhibition Zone (mm)			HSD at 0.05
	U. urens extract	Se/CuO NPs	Control (Ampicillin /Nystatin)	
P. glabrum	0.0±0.00^{b}	0.0±0.00^{b}	30±1.23^{a}	3.26
C. albicans	25±0.65^{c}	31±0.48^{a}	28±0.32^{b}	2.65
E. coli	26±1.25^{a}	28±0.58^{a}	24±0.87^{b}	2.08
S. typhi	19±1.12^{b}	26±0.69^{a}	21±1.23^{b}	3.21
B. subtilis	24±0.98^{b}	26±0.54^{a}	24±0.87^{b}	0.94
S. aureus	25±0.28^{a}	27±0.74^{a}	23±1.01^{b}	2.29

Both MIC and MBC were assayed to characterize Se/CuO NPs and *U. urens* extract, and the results are shown in Table 2. The experiment reflected that MIC and MBC of Se/CuO NPs was less than *U. urens* extract with different levels. Excellent quantity of MBC (15.62±3.54 µg/mL) was recorded using Se/CuO NPs compared with its quantity (125±2.65 µg/mL) using extract in case *S. typhi*.

The same excellent result was recorded with the calculation of MBC in case *S. typhi*, where the MBC of the Se/CuO NPs exhibited was 31.25±2.54 µg/mL compared with 500±3.65 µg/mL MBC of the extract. Other outcomes were illustrated in Table 2. These results show the properties of both Se/CuO NPs and *U. urens* extract towards the tested microorganisms. The effect of both Se NPs and CuO NPs *versus* growth of some bacteria was documented with MIC values of 125 µg/mL against *S. aureus* and 100 µg/mL against *E. coli* (Rasheed *et al.* 2024).

Antioxidant Activity of Se/CuO NPs and *U. urens* Extract

From the results of antioxidant activity, it is clear that both Se/CuO NPs and *U. urens* extract possessed the highest capacity for radical scavenging with little difference (Table 3); however, the obtained IC_{50} values indicated that Se/CuO NPs (4.2±0.41 µg/mL) were more effective than the *U. urens* extract (5.12 ±0.19 µg/mL). In parallel, the activity of ascorbic acid was examined as antioxidant control, which gave an IC_{50} of 2.28±0.16 µg/mL. In another study (Dulta *et al*. 2022), antioxidant activity of CuO NPs was documented by with IC_{50} of 91.2 µg/mL.

Many investigations demonstrated that NPs possess more antioxidant potential than the extract of plant, which was employed for its synthesis (Sentkowska and Pyrzyńska 2023). Antioxidant activity may depend on the natural mediator of NPs synthesis. The CuO NPs were found to possess antioxidant activity with IC_{50} of 28.05 µg/mL, while 51.71 µg/mL was associated with their producer *Suaeda maritime* (Peddi *et al*. 2021). Published papers documented the antioxidant activity of *U. urens* extract *via* DPPH, which is in agreement with the authors' investigations Mzid *et al*. (2017) and Salem *et al*. (2021).

Anti-hemolysis Activity of Se/CuO NPs and *U. urens* Extract

Destruction of human as well as animal red blood cells is known as hemolysis, causing liberation of hemoglobin. In the current work, management of hemolysis by Se/CuO NPs and *U. urens* extract compared with Indo (positive control) was recorded in Table 4 and Fig. 4. The hemolysis inhibition increased with the applied concentration increased up to 1000 µg/mL of both tested substances.

Table 2. Effective of *U. urens* Extract and Se/CuO NPs MIC and MBC with its MIC/MBC Index

Microorganism	MIC µg/mL		HSD at 0.05	MBC µg/mL		HSD at 0.05	MBC/MIC index		HSD at 0.05
	U. urens extract	Se/CuO NPs		*U. urens* extract	Se/CuO NPs		*U. urens* extract	Se/CuO NPs	
C. albicans	62.5±2.01a	15.62±1.25b	5.32	250±3.24a	62.5±2.15b	6.86	4±0.12a	4±0.09a	0.21
E. coli	31.25±1.23a	7.8±0.95b	3.26	125±2.10a	31.25±1.52b	5.95	4±0.25a	4±0.19a	0.16
S. typhi	125±2.65a	15.62±3.54b	6.32	500±3.65a	31.25±2.54b	7.65	4±0.35a	2±0.42b	0.56
B. subtilis	31.25±1.02a	15.62±1.36b	2.45	62.5±1.65a	15.62±2.15b	2.58	2±0.21a	1±0.29b	0.18
S. aureus	31.25±1.32a	15.62±2.26b	3.85	62.5±2.32a	15.62±0.98b	4.36	2±0.14a	1±0.08b	0.24

Table 3. Antioxidant Activity of Ascorbic Acid Se/CuO NPs and *U. urens* Extract

Concentration (µg/mL)	***U. urens* extract**	**Se/CuO NPs**	**Ascorbic Acid**	**HSD at 0.05**
Control 100%	0.0	0.0	0.0	000
1.95	39.0±1.02b	40.8±0.65b	44.8±0.84a	2.12
3.9	44.7±1.20b	47.9±1.06b	54.1±0.87a	3.65
7.81	54.3±1.29b	55.2±0.52b	60.0±1.65a	2.02
15.62	62.1±0.88b	62.9±1.02b	67.5±0.54a	1.65
31.25	69.1±1.03b	70.6±2.15b	75.4±1.87a	2.32
62.5	76.2±0.65b	77.9±0.54b	84.2±1.28a	3.03
125	81.4±0.65c	84.6±0.48b	91.5±1.10a	3.01
250	88.9±0.58c	92.0±1.07b	94.1±0.67a	2.45
500	93.0±1.21b	94.2±0.32b	96.4±0.59a	1.36
1000	95.2±0.21b	96.5±0.14b	98.1±0.25a	2.98
IC_{50} µg/mL	5.12 ±0.19 a	4.2±0.41a	2.28±0.16c	0.69

Surprisingly the *U. urens* extract exhibited activity for preventing the hemolysis that was higher than Se/CuO NPs. Moreover, a similar effect (non-significant) was associated with the extract and Indo at all examined conditions on the hemolysis inhibition. These descriptions were confirmed *via* calculation of IC_{50}, where *U. urens* extract, Se/CuO NPs, and Indo inhibited the hemolysis at IC_{50} of 4.1±0.12, 12.26±0.22, and 4.00±0.36 µg/mL, respectively. Blood hemolysis was observed in the red blood cells that were exposed to CuO NPs (Pourahmad *et al.* 2023). Dhabian and Jasim (2023) also showed the anti-hemolytic activity of Se NPs.

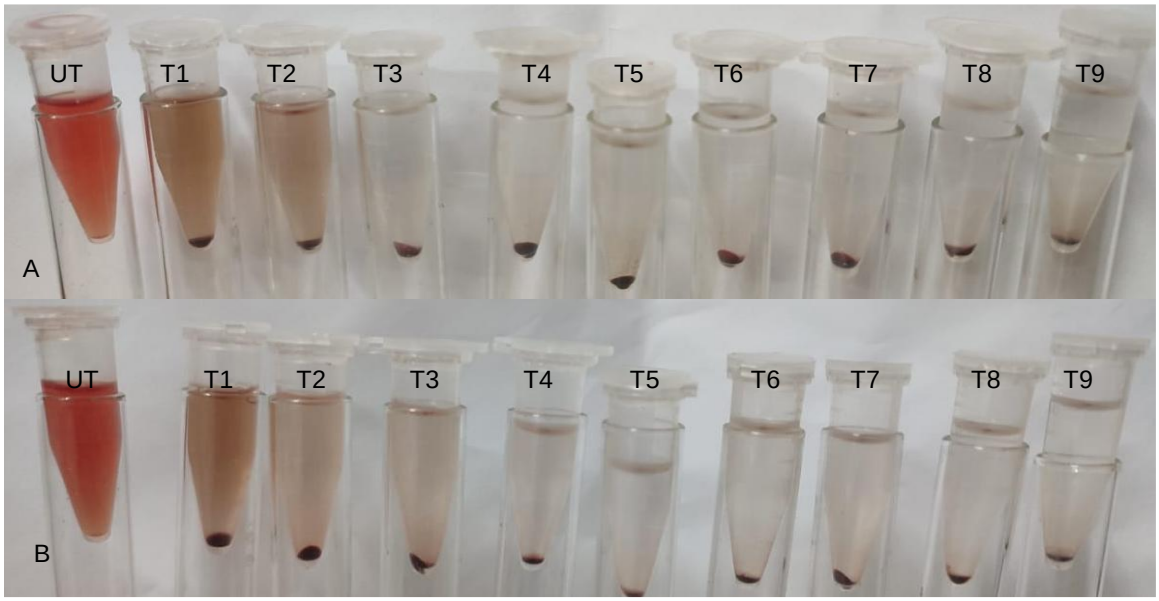

Fig. 4. Hemolysis inhibitio**%** of Se/CuO NPs (A) and *U. urens* extract (B) at different concentrations. T1 to T8 3.9 to 1000 µg/mL, T9 treated with Indomethacin, UT untreated sample

Table 4. Hemolysis Inhibition% of Se/CuO NPs and *U. urens* Extract and Indomethacin

Concentration (µg/mL)	Hemolysis Inhibition**%**			HSD at 0.05
	U. urens extract	Se/CuO NPs	Indomethacin	
Control 100%	0	0	0	000
3.9	43.4±1.25^{a}	32.9±0.95^{b}	44.7±0.36^{a}	3.211
7.81	53.8±1.65^{a}	42.9±1.26^{b}	54.5±1.11^{a}	5.125
15.62	64.9±0.42^{a}	52.6±0.25^{b}	65.1±1.02^{a}	2.332
31.25	74.3±0.65^{a}	62.9±1.03^{b}	73.4±0.88^{a}	3.201
62.5	83.3±0.12^{a}	72.9±0.41^{b}	81.7±1.03^{a}	1.325
125	90.0±0.52^{a}	80.9±0.21^{c}	87.9±0.15^{b}	2.265
250	92.1±0.45^{a}	85.4±0.14^{b}	93.0±0.21^{a}	1.650
500	96.9±0.65^{a}	89.2±0.41^{b}	95.8±1.00^{a}	1.562
1000	98.1±0.45^{a}	92.2±0.36^{b}	98.9±0.54^{a}	0.952
IC_{50} (µg/mL)	4.1±0.12^{a}	12.26±0.22^{b}	4.00±0.36^{a}	0.321

Anticoagulation Activity Se/CuO NPs and *U. urens* Extract

Anticoagulation of Se/CuO NPs and *U. urens* extract compared with heparin at different concentrations was recorded in Table 5. Se/CuO NPs exhibited significantly greater anticoagulant potential than the *U. urens* extract but not the same for heparin. At

25 µg/mL, the prothrombin time (PT) was 17.4±0.96, 20.32±1.21, and 25.14±1.65 S; the partial thromboplastin time (PPT) was 35.45±0.09, 39.2±0.19, and 66.1±0.08 S; while at 75 µg/mL the PT was 45.3±0.10, 59.12±0.21, and 99.80±0.33 S; PPT was 73.4±0.21, 98.1±0.15, and 145.7±0.11 S of *U. urens* extract, Se/CuO NPs, and heparin. According to Ahmed *et al*. (2021), increasing the clotting time was observed with management of CuO NPs. The developed anti-coagulant effect may be due to inhibition of thrombin, as well as intrinsic coagulation factor as mentioned previously by Di Cera (2008). In a recent investigation (Siddique *et al*. 2024), several pharmacological utilizations were associated to Se NPs, such as anticoagulants, antioxidant, and thrombolytic potentials. In an *in vitro* study, Omar *et al*. (2017), showed that the *U. urens* extract possess either coagulation or anticoagulation influence on the tested blood, which is a result that matched our findings.

Table 5. Anticoagulant Activity at Different Doses of Se/CuO NPs, *U. urens* Extract and Heparin via Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT)

Dose (µg/mL)	PT (Sec)			HSD	PTT (Sec)			HSD
	U. urens extract	Se/CuO NPs	Heparin		*U. urens* extract	Se/CuO NPs	Heparin	
0.0	12.5 ±0.23a	12.5 ±0.32a	12.50 ±0.15a	1.68	30.0 ±1.21a	30.0 ±0.95 a	30.0 ±1.21a	5.21
25	17.4 ±0.96c	20.32±1.21b	25.14 ±1.65a	2.32	35.45 ±0.09c	39.2 ±0.19b	66.1 ±0.08a	3.25
50	24.8 ±0.22c	32.1±0.09b	49.80 ±0.21a	4.02	50.4 ±0.06c	61.6 ±0.32b	93.8 ±0.18a	2.23
75	45.3 ±0.10c	59.12±0.21b	99.80 ±0.33a	3.26	73.4 ±0.21c	98.1 ±0.15b	145.7 ±0.11a	6.32

Effect of bimetallic Se/CuO NPS on Morphological Traits and Pigment Contents of Maize Plant

The tested plant, the created NPs (dose, particle size, and shape), and the method of application (foliar, substrate, and seeds) all were found to influence the differing effects of NPs on plants. In this study, different doses of Se/CuO NPS were used to improve growth and pigment contents of maize plants. Based on data in Table 6, it appeared that bimetallic Se/Cu NPS at 1, 5, 10, 20, 50, and 200 ppm exhibited significant improvement in shoot lengths of maize plant. The highest increase of shoot and root lengths occurred in the 10 ppm treatment by 32.6 % and 17.9%, respectively, compared to the control. Se/CuO NPS at 20 ppm caused the highest value of fresh weight of shoot and root. In the same concept on tomato plants, Hernández-Hernández *et al*. (2019) found that, adding 10 mg/L of Se NPs could boost tomato output by as much as 22 percent. Additionally, Shafiq *et al*. (2024) found that CuO NPs improved growth traits of growth and biochemical characteristics of maize grown under normal conditions or stress conditions of salinity.

In the photosynthetic system, chlorophylls play a critical role that is strongly correlated with plant biomass and recovery. Data in Fig. 5 appeared to show that Se/CuO NPS at 1, 5, 10, 20, 50, and 100 ppm led to significant improvement in pigments content (chlorophyll a, b and carotenoid) of maize plant compared to the control and other treatments. The treatment of 10 ppm resulted in the highest significant improvement in chlorophyll a and b; the highest value of carotenoids occurred after treatment of 20 ppm of bimetallic compared to the control and other treatments.

Table 6. Morphological Traits of Maize Plant under Different Concentrations of Se/CuO NPS

Concentration	Shoot length	Root length	Fresh weight of shoot	Fresh weight of root
Control	43.60±5.03b	10.40±2.97a	7.888±1.318c	1.0260±0.2033bc
1 mg	54.40±3.87a	8.720±1.057a	10.338±2.215abc	1.136±0.1696abc
5 mg	52.20±3.91ab	10.558±1.606a	10.036±1.128abc	1.0020±0.1464c
10 mg	57.30±3.44 a	12.26±2.99a	11.726±1.583ab	1.552±0.401ab
20 mg	52.40±7.44ab	12.08±2.31a	13.128±1.049a	1.6420±0.1038a
50 mg	53.90±4.23a	9.40±3.86a	10.90±2.83bc	1.4060±0.1996abc
100 mg	49.80±3.87ab	11.080±1.219a	8.588±1.305bc	1.160±0.224abc
200 mg	51.71± 1.30ab	9.60±3.65a	11.402±1.090b	1.096±0.467abc
HSD at 5%	8	4.65	3.084	0.486

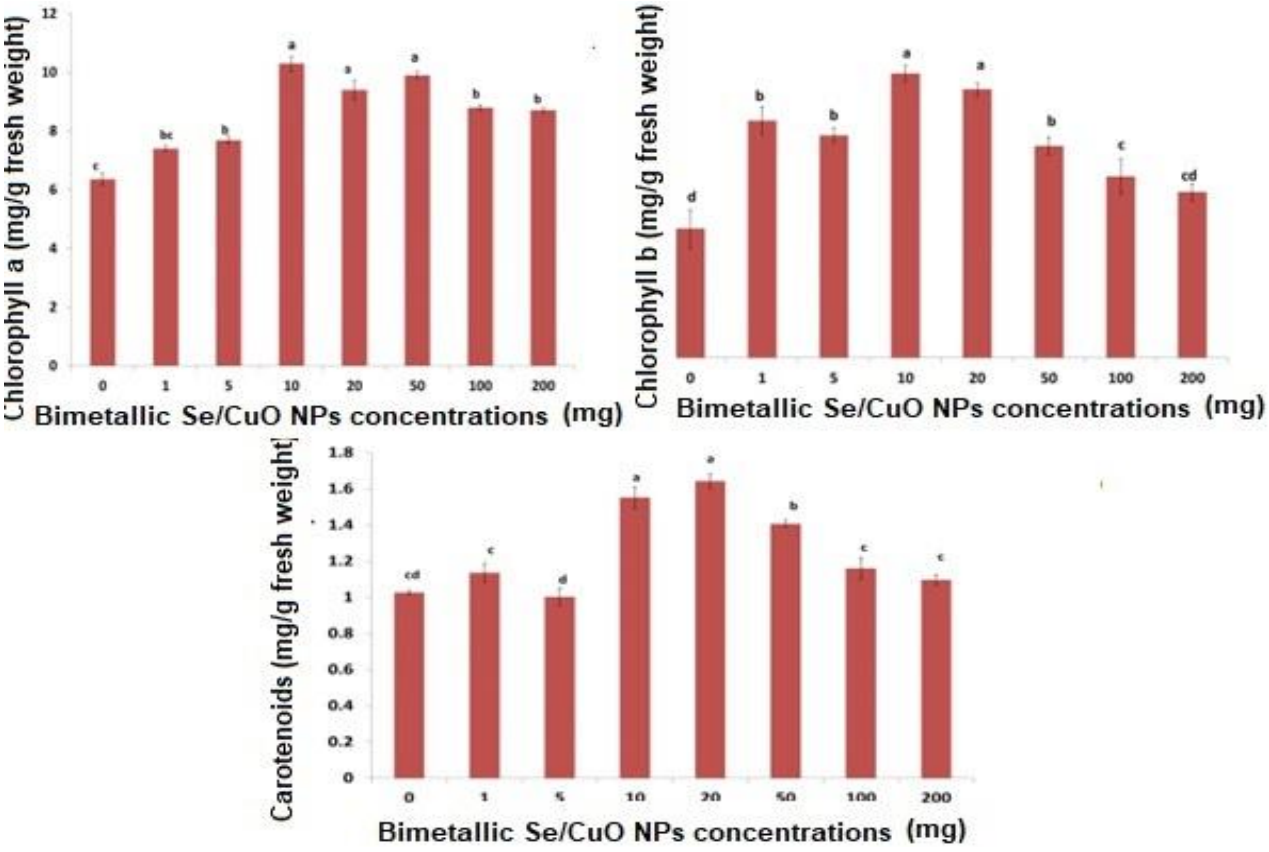

Fig. 5. Effect of different concentrations of Se/CuO NPs on pigments contents of maize plant. The standard deviation of the means plus the mean of three replicates make up each number. Values of the same letter in the same column do not differ significantly; nevertheless, post hoc Tukey's test results at $P < 0.05$ show significant differences between distinct lower-case letters in the same column

Hernández-Hernández *et al*. (2019) found that the application of Cu at 10, 50, and 250 ppm and Se at 1 and 10 ppm increased chlorophyll content of tomato plants. Also, González-García *et al.* (2021) showed that application of Se NPs 10 and 50 mg L^{-1}, Cu NPs 100 and 500 mg L^{-1} caused increased chlorophyll in the leaves of bell pepper, which minimize the degradation of chlorophyll in the plant. Furthermore, certain chloroplast enzymes involved in the manufacture of photosynthetic pigments are positively impacted

by NPs. According to Hussein *et al*. (2019), the application of Se NPs increased the amount of chlorophyll in the peanut crop. Furthermore, certain chloroplast enzymes included in the making of photosynthetic pigments were positively affected by NPs, and as a result chlorophylls and other pigments may accumulate in the leaves.

CONCLUSIONS

1. *Urtica urens* extract showed success in an eco-friendly procedure for synthesizing Se/CuO nanoparticles (NPs).
2. Se/CuO NPs were found to be particularly effective against pathogenic microorganisms including C. *albicans*, *E. coli*, *S. typhi*, *B. subtilis*, and *S. aureus*.
3. Se/CuO NPs exhibited antioxidant properties, and the ability to scavenge free radicals was documented.
4. Anti-hemolysis of *U. urens* extract was higher than Se/CuO NPs, while the anticoagulant potential of Se/CuO NPs was recorded as better than the *U. urens* extract *via* partial thromboplastin time (PPT) and prothrombin time (PT).
5. The current study has shown that there is a significant improvement of morphological traits and pigments contents of maize plant.

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